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# ASSESSMENT OF PATHOLOGICAL CHANGES OF MIXED INFECTION OF COCCIDIOSIS AND NECROTIC ENTERITIS IN TURKEY

By

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## ABSTRACT

Necrotic enteritis is a great problem in poultry industry globally. Little information exists concerning the pathogenesis of necrotic enteritis in turkeys. Bacteriological and parasitological examination of 100 intestinal samples revealed that 55%, 39% and 34% had C. *perfringens*, *Eimeria* species and mixed infection, respectively. Investigation of toxigenic subtypes among the isolates by multiplex PCR showed that (53.8%) of studied isolates considered as *C. perfringens* type A as harbored *cpa* gene only. Antimicrobial susceptibility results demonstrated that, the highest resistance against colistin (89%). Amoxicillin was most effective against 45 (81.8%) tested isolates of *C. perfringens*. Therefore, the present work was designed to develop an experimental model of necrotic enteritis and coccidiosis in turkey to study the growth performance, biochemical parameters and histopathological changes associated with it. Seventy, one day old turkeys were divided into seven equally groups, group (1) negative control. Group (2, 3 and 4) was infected orally with *Eimeria* oocysts, *C. perfringens* and both *Eimeria* oocysts and *C. perfringens*, respectively. Group (5) was treated with diclazuril after infection with oocysts of Eimeria. Group (6) was treated with amoxicillin post infection with *C.perfringens*. Group (7) was treated with (diclazuril+amoxicillin) after infection with both *Eimeria* oocysts + *C. perfringens*. Experimental study revealed disturbance in proteingram, lipogram and liver and kidney function test detected throw the biochemical and pathological changes. The amoxicillin and diclazuril had significant improvement in growth performance, in addition to reduction in mortalities lowered number of oocysts, reduce the severities of necrotic enteritis (NE) and clostridia count in addition to amelioration of the biochemical and pathological changes.

**Keywords:**

*C. perfringens* - *Eimeria* spp - Multiplex PCR - Growth performance - Pathological changes- Serum chemistry.

## INTRODUCTION

Necrotic Enteritis (NE) and coccidiosis are a wide spread diseases of considerable economic importance to the turkey industry, as it causes high losses among birds in addition to the high cost of its control **(Timbermont *et al.*, 2009)**. Avian necrotic enteritis was first described in 1961 and since then it has been reported to occur in almost all poultry-producing countries **(Mcdevitt *et al*., 2006)**.

The *C. perfringens* produces at least 12 different toxins, which are associated with the occurrence of NE. Not all the *C. perfringens* inhabiting the gut pathogenic and only few of the strains are virulent and pathogenic for major extracellular toxin types, namely alpha (α), beta (β), epsilon (ε), and iota (i), are produced by biotypes of *C. perfringens* A, B, C, D, and E **(Paiva and McElroy, 2014)**.The α -toxin was the major toxin involved in necrotic enteritis in poultry **(Timbermont *et al.*, 2009)**.

The disease result in outbreaks with mortality rates up to 50% as an acute enterotoxaemia. The clinical illness is usually of rapid onset, and often the only signs are a severe depression followed quickly by a sudden increase in flock mortality. The disease primarily affects broiler chickens (2-5 weeks old) and turkeys (7-12 weeks old) **(Osman and Elhariri, 2013).** For many years, prophylactic use of antibiotic in feed has been primary means of controlling necrotic enteritis in broiler industry. However development of *C. perfringens* strain resistance to antibiotic has threatened economic stability of broiler industry **(Baumgartner, 2003).** Adequate antibiotic treatment in the early stages of the disease may control the infection **(Lyras *et al.*, 2009).**

One of the common predisposing factors of NE is a coccidiosis infection **(Paiva and McElroy**, **2014)**. Coccidiosis is an important disease of the turkey caused by protozoan Parasites of the genus Eimeria; the intestinal damage caused by coccidia is an essential predisposing factor for NE resulting in over growth of *C. perfringens* and toxin production **(Assis *et al.*, 2010)**. Anticoccidia compounds should be highly effective against all developmental stages of *Eimeria* species, don't effect on the host immune response as well as have no residues in the tissues. In this respect, diclazuril is one of a series of benzenacetonitrile derivatives; its efficacy of in feed was studied in turkey **(Chapman *et al.*,**

**2004).** Lamina propria of intestine is infiltrated with inflammatory cells leading to an extensive disorder of intestinal integrity **(Olkowski *et al.*, 2008)**. In vivo experimental models of necrotic enteritis in turkeys, and a basis for acquisition of new knowledge about the pathogenesis, immunity and other important aspects of this disease **(Hardy *et al.*, 2020)**.

The objective of this study is to investigate the incidence of mixed infection of C. *perfringens* and *Eimeria* species in turkeys to study antimicrobial **s**usceptibility profiles of C. *perfringens* and screen toxigenic attributes of circulating strains by multiplex PCR methods and to identify the experimental infection effect of coccidiosis and necrotic enteritis on growth performance, serum chemistry and histopathological changes, find out the efficacy of diclazuril and amoxicillin on the experimentally affected turkeys.

## MATERIAL AND METHODS

**Sampling and isolates characterization.**

**Collection of Samples:** One hundred intestinal samples (jejunum and ileum) were collected from different turkey farms of different species aged from (4-12 weeks) with history of mortalities, depression, growth retardation and diarrhea were used for isolation *C. perfringens*. The same intestinal content was used for isolation of *Eimeria* species. All samples were collected randomly from different localities of El-Sharkia Governorate, Egypt in clean, dry and sterile containers then transferred to the laboratory as soon as possible to be examined.

**Isolation and identification of *Clostridium perfringens*:**

Each sample was inoculated into a tube containing freshly prepared cooked meat broth medium (Oxoid, UK). The tube was incubated anaerobically at 37°C for 24 hours using anaerobic jar, then was streaked onto the surface of 10% sheep blood agar (Oxoid, UK), supplemented with neomycin sulphate at a concentration of 200µg/ml according to **Carter and Cole (1990**). All plates were incubated anaerobically at 37°C for 24 hours. Suspected colonies of *C. perfringens* initially characterized by double zone hemolysis were picked up and maintained in cooked meat broth and identified microscopically after Gram stain which showed Gram positive bacilli. Afterwards, colonies were subsequently characterized using biochemical tests including catalase test, sugar fermentation test and indole test as previously published by **Koneman *et al.*, (1988).**

**Isolation of *Eimeria* species**.

Mucosal scraping was examined for detection of *Eimeria* species oocysts by floatation concentration technique with saturated sodium chloride by **Permin and Hansen (1998).** Furthermore, oocysts were collected directly from the infected birds through lesion scraping. After examination positive samples were strained through sieve and put into petri-dish contain potassium dichromate solution (2.5%) to allow sporulation at room temperature for 7 days. The collected oocysts washed by distilled water 3-4 times and centrifuged on 3000 rpm for 10 minutes to remove the potassium dichromate and stored at 4ºc.

**Antimicrobial susceptibility testing:**

*In vitro* susceptibility testing of isolates was applied by agar disk diffusion method according to British Society for Antimicrobial Chemotherapy **(BSAC, 2011).**

The susceptibility testing were applied against ten antimicrobial agents of the commonly used in the field; amoxicillin (AX:10 µg), amikacin (AK:30 µg), bacitracin (B:10 µg), lincomycin (L:30 µg), sulfamethoxazole/trimethoprim (SXT: 25 µg) neomycin (N:30 µg), cefotaxime (CTX:30 µg), enrofloxacin (ENR:5 µg), clindamycin (DA:2 µg) and colistin (CT: 10 µg ) by using commercial disks from Oxoid laboratories. Antimicrobial susceptibility tests were carried out on 10 % sheep blood agar medium in order to support the growth of anaerobic bacteria **(Perelman *et al.*, 1991)**. The inhibition zone was measured for each antibiotic and resistance breakpoints were determined according to BSAC methods for antimicrobial susceptibility testing (Version 10.2, **2011**).

**Molecular characterization of *Clostridium perfringens.***

**Tested isolates:**

A total of thirteen *C. perfringens* isolates were used for DNA extraction that showed the highest multidrug resistance (MDR).

**DNA extraction:** QIAamp DNA Mini kit (Qiagen, Germany, GmbH) was used.

**Oligonucleotide primer:** Primers were supplied from Metabion (Germany), primers' sequences; thermal profiles for PCR were shown in (Table 1).

**PCR amplification:**

**Multiplex PCR for toxins,** primers were utilized in a 50- µl reaction containing 25 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentrations, 11 µl of water, and 6 µl of DNA template. The reaction was performed in an applied biosystem 2720 thermal cycler, Germany. The products of PCR were separated by

electrophoresis on 1.5% agarose gel **(Applichem, Germany, GmbH).**The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

**Drugs:**

**Anticoccidia drugs:** Diclazuril 10mg: Diclosol (Pharma Swede Company) dose: 1ml/2liters drinking water for two days.

**Antibiotics:** Amoxicillin antibiotic powder: Amoxitryl (Pharma Swede Company) dose: 20mg /kg body for 3 days.

**Experimental design:**

A total number of 70 turkeys native breed (Balady turkey) at one day age were reared; feces of birds were examined to confirm the absence of coccidia from one day old till 21$^{th}$ day old using salt flotation **(Permin and Hansen, 1998)**. Turkeys were divided randomly into seven equally groups, birds in all groups were supplied with drinking water and starter feed (28% protein) ad-libitum. Turkeys of group (1) remain as non-infected non- treated (negative control). Group (2) was infected orally at 21day old with 100.000 sporulated oocysts of *Eimeria* spp **(Dalloul *et al.*, 2003)**. Group (3) infected orally at day 28$^{th}$ day old with 2.5ml of $10^8$ CFU *C. perfringens* inoculums **(Ferdoush *et al.*, 2014).** Group (4) infected with both *Eimeria* spp (21$^{th}$day old) and *C. perfringens* (28$^{th}$ day old). Group (5) was treated with diclazuril after infection with oocysts of *Eimeria* spp. Group (6) was treated with amoxicillin days starting from the third day post infection with 2.5ml of $10^8$ CFU *C. perfringens* inoculums **(Abd El-Hamid *et al.*, 2015)**. Finally Group (7) was treated with (diclazuril+amoxicillin) after infection with both *Eimeria* spp + *C. perfringens* with the same doses and duration.

**1. Performance parameters:**

The clinical symptoms appeared post infection and number of dead bird were recorded, body weight and body weight gain of each group weighed at day of infection and weighed at one week and two week post infection, feed conversion was calculated as **Wagner *et al.*, (1983).**

$$\text{F-conversion rate} = \frac{\text{Feed consumption (g) period}}{\text{Weight gain (g) period}}$$

**Sampling:**

**Blood samples:** Blood samples from five poults in each group were collected at the end of treatment period from the wing vein under aseptic conditions without anticoagulant for the separation of sera. The sera samples were stored at freezer - 20°C for further biochemical analysis.

**Tissue samples:** Specimens from the intestine, liver, and kidney were collected in 10% formalin for histopathological examinations.

**2. Biochemical studies:**

Total protein (TP) level and albumin level were determined by **Krohn (2005)** and **Pinnell and Northam (1978)**, respectively and serum globulin level was calculated by subtracting the obtained albumin level from the TP level. Protein electrophoresis was performed as **Henery *et al.*, (1974)**. The activities of serum ALT , AST, ALP, calcium, and inorganic phosphorus were determined as **Tietz (1995)**, serum cholesterol, High-density lipoprotein (HDL) **(Naito 1984),** low density lipoprotein (LDL) and very density lipoprotein (VLDL) **(Nauck *et al.*, 2002)**. Serum triglyceride concentration **(Mamoru *et al*., 1977)**.

**3. Pathological examination:**

The collected specimens from intestine, liver and kidney were fixed in 10% neutral buffer formalin then processed using the routine histopathological technique and stained with haematoxylin and eosin stain and examined microscopically **(Suvarna,2018).**

The histopathological lesion grading was calculated by description of changes in 5 fields per section for each examined organ **(Katherine *et al*., 2013).**

**4. *C. perfringens* and oocysts counts.**

Concerning *C. perfringens* approximately 1-2 gram of intestinal contents from each of five birds from the infected groups of turkey were collected; samples from each group were pooled for bacterial re-isolation and count 7 days post infection **(Soad *et al.*, 2015)**. Count was expressed as log10 CFU per gram of intestinal contents, **(Cruickshank *et al.*, 1975)**. Isolated colonies were then biochemically tested, Gram stained and microscopically examined to be confirmed as *C. Perfringens*. The oocysts were counted using the McMaster counting chamber technique as described by **Long *et al.*, (1976).**

**5. Statistical analysis.**

The data in this study were statistically analyzed by one way anova **(Tamhane and Dunlop, 2000)** using the MSTAT-C computer program. Results are presented as mean ± SE, and the

statistical significance was set at (P≤0.05).The significance between groups represented by small letters and the highest value represented by (a) letter.

**Table (1):** Primers sequences, amplicon sizes and cycling conditions.

| Target gene | Primers sequences (5'-3') | Amplifi-ed segment (bp) | Primary denaturation | Amplification (35 cycles) | | | Final extension | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | Secondary denaturation | Annealing | Extension | | |
| Alpha | GTTGATAGCGCAGG ACATGTTAAG<br>CATGTAGTCATCTG TTCCAGCATC | 402 | 94°C 5 min. | 94°C 45 sec. | 55°C 45 sec. | 72°C 45 sec. | 72°C 10 min. | YOO *et al.*, 1997 |
| Beta | ACTATACAGACAGA TCATTCAACC<br>TTAGGAGCAGTTAG AACTACAGAC | 236 | 94°C 5 min | 94°C 45 sec. | 55°C 45 sec. | 72°C 45 sec | 72°C 10 min. | |
| Epsilon | ACTGCAACTACTAC TCATACTGTG<br>CTGGTGCCTTAATA GAAAGACTCC | 541 | 94°C 5 min. | 94°C 45 sec. | 55°C 45 sec. | 72°C 45 sec | 72°C 10 min. | |
| Iota | GCGATGAAAAGCCT ACACCACTAC<br>GGTATATCCTCCAC GCATATAGTC | 317 | 94°C 5 min. | 94°C 45 sec. | 55°C 45 sec. | 72°C 45 sec | 72°C 10 min. | |

## RESULTS

**Incidence of *C. perfringens* and *Eimeria* spp.**

*C. perfringens* was isolated from 55 of 100 pooled intestinal samples (jejunum and ileum) (55%). They were identified by standard microbiological techniques. With regard to *Eimeria* species it was found in (39%) that was identified from turkey suffering from bloody coccidiosis. From examined samples 34% had mixed infection of *C. perfringens* and *Eimeria* species.

**Antimicrobial susceptibility testing.**

All *C. perfringens* isolates expressed resistance to the most used antimicrobial agents. Antimicrobial sensitivity profiling of the 55 confirmed *C. perfringens* isolates, indicated that,

higher rates of sensitivity to amoxicillin was 45 (81.8%) followed by cefotaxime (71 %) and clindamycin (69.1%). On the other hand, out of 55 isolates, (89%) were found resistant to colistin followed by (72.7%) to neomycin and (63.6%) to sulfa-methanol / trimethoprim (Table 2). Overall, results showed that most isolates were resistant to at least three of the tested antimicrobial agents, making them multidrug resistant (MDR).

**Table (2):** Phenotypic antimicrobial susceptibility profiles of *C. perfringens* isolates.

| Antimicrobial agent | *C. perfringens* (55) | |
|---|---|---|
| | Resistant (%) | Sensitive (%) |
| Colistin (CT) | 49(89%) | 6 (11%) |
| Neomycin (N) | 40 (72.7%) | 15 (27.2%) |
| Sulfamethoxazole/trimethoprim (SXT) | 35 (63.6%) | 20 (36.3%) |
| Enrofloxacin (ENR) | 28 (51%) | 27 (49%) |
| Amikacin (AK) | 22 (40%) | 33 (60%) |
| Lincomycin (L) | 18 (32.7%) | 37 (67.2%) |
| Bacitracin (B) | 17 (31%) | 38 (69.1%) |
| Clindamycin (DA) | 17 (31%) | 38 (69.1%) |
| Cefotaxime (CTX) | 16 (29%) | 39 (71%) |
| Amoxicillin (AX) | 10 (18.1%) | 45 (81.8%) |

**Detection of toxigenic attributes of *C. perfringens* isolates by PCR.**

Toxino typing of isolates by PCR was applied on 13 randomly selected MDR C. *perfringens* isolates. PCR targeted the detection of toxigenic genes *cpa* gene encodes for alpha toxin, *cpb* gene encodes for beta toxin, *εtx* gene encodes for epsilon toxin and *i*A gene encodes for iota toxin, respectively. The results revealed that 7/13 (53.8%) of PCR tested isolates belonged to *C.perfringens* type A and produced positive PCR result for only *cpa* gene that encodes for alpha toxin with the production of specific amplicon at 402 bp. On the other hand, none of the 13 tested isolates was positive for *cpb* gene, *etx* gene, or *i*A gene as failed to produce the relevant specific amplicon at 236 bp, 541 bp and 317bp, respectively Fig. (1).

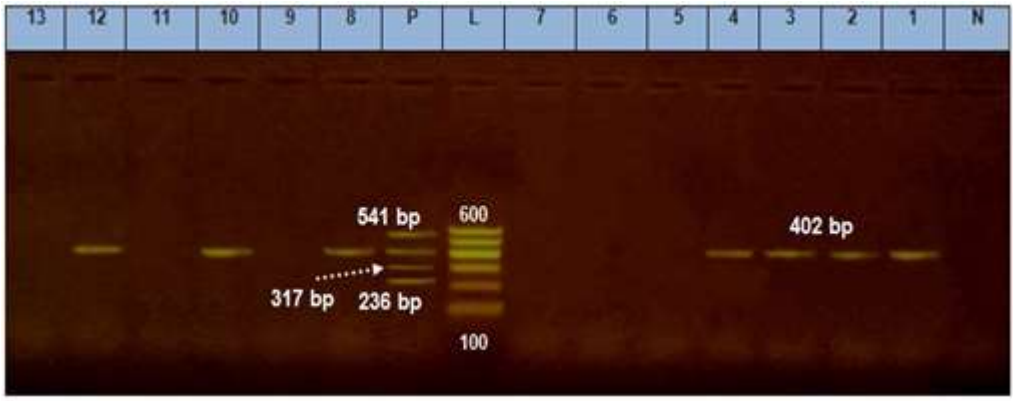


**Fig. (1):** Multiplex PCR for Toxino typing of *C.perfringens* isolates. Lane Neg.: negative control; (*E.coli*). lane POS: Positive control for C.*perfringens* type A with positive PCR result for *cpα*, *cpβ*, *εtx* genes and *i*A genes encode for alpha, beta, epsilon and iota toxins, with production of specific amplicons at 402 bp, 236 bp 541 bp and 317 bp, respectively, lane L: gene ruler ladder 100-1000 bp. Lanes 1, 2, 3, 4, 8, 10, 12: positive for *cpa* gene encodes for alpha toxin with production of specific amplicon at 402 bp.

**Clinical signs, postmortem lesions and count of *Eimeria* spp and *C. perfringens*.**

The clinical signs observed in infected groups (2, 3 and 4) were depression, ruffled feather, bloody diarrhea and growth retardation. With regard to mortalities, the results showed that group (4) mixed infection with (coccidia + *C.perfringens*) had higher mortality (50%) of birds than group (2) infected with coccidian had (40%) mortality with regard to macroscopic lesions there was dilation, hemorrhage in upper intestine and mucoid exudates tinged with blood in caecum. Furthermore, group (3) infected with *C. perfringens* had (30%) mortality, with macroscopic lesion showed that small intestine was thin, dilated wall and filled with gas (Ballooning of intestine) and covered with diphteric membrane. While groups (5), (6) and (7) were treated with diclazuril, amoxicillin, and (amoxicillin+ diclazuril), respectively recorded mortality (20%) for all these groups

Upon oocysts count group (1) the oocyst not detected as well as there was decrease in the number of oocysts shedding in infected treated groups 5 and 7 ($4X10^3$), ($3.2\ X10^3$), respectively, as compared with control group (2) ($6.4\ X10^4$).

Concerning clostridia enumeration, group (1) that was control negative showed clostridia count ($4X10^4$ CFU/ml). Group (6) treated with amoxicillin had lower clostridia count ($5X10^5$ CFU/ml) compared to positive control group (3) which had ($1.5X10^9$ CFU/ml) count**.**

The group (4) infected with both *C. perfringens* and *Eimeria* spp (mixed group) had highest clostridia count ($6X10^9$) compared to treated group (7) with count ($4X10^5$ CFU/ml).

There was significant reduction in B.W, B.W gain, F consumption and higher FCR in the infected non treated groups (2,3,4) as (1101.67 ±7.26 , 310 ±15.27, 560 ± 5.77 and 1.88 ±0.09 ), (1090 ±5.77, 280 ±2.89, 533.33 ± 8.82 and 1.84 ±0.03) and (965 ±2.89 , 231.67 ±6.01, 450±11.55 and 1.97 ±0.04), respectively as compared with control group (1) (1531.67 ±6.0, 511.67 ±6.01 , 790± 11.55 and 1.53 ±0.03) (Table 3).

The treated groups (5, 6 and 7) showed significant improvement in (BW), (BW gain). Food consumption and FCR were as, (1306.67 ±4.41 , 393.33 ±8.82 , 650±5.77 and 1.62 ±0.03 ), (1313.33 ±12.02 , 396.67 ±6.01, 670±11.55 and 1.63 ±0.02 ) and (1315 ±2.89 , 403.33 ±8.82 , 666.67± 8.82 and 1.61 ±0.04 ), respectively as compared with infected groups (Table 3).

**Biochemical changes.**

A significant decrease ($P\leq0.05$) in total protein and albumin in groups (2, 3, 4 and 5) compared with normal control group but no significant change in groups (6 and 7). The A/G showed significant decrease in groups (2, 3, 4, 5, and 6). The total globulins and their fractions showed significant increase ($P\leq0.05$) in groups (3 and 4) except beta 2 globin showed significant decrease in all groups and alpha 2 globin significantly increased in all groups. Significant increase in the serum activity of ALT, AST and ALP in groups (3 and 4) compared to the control group and no significant changes on their activity on other groups. The triglycerides level significantly increased in groups (2, 3 and 4) only in comparison with the normal control group but the total cholesterol level significantly increased in groups (3 and 4) only. In addition to this the HDL level showed significant decrease in all groups, the LDL level showed significant increase in groups (3 and 4) and the VLDL level showed significant increase in groups (2, 3, 4 and 5) other groups revealed nonsignificant changes in the other lipogram parameters when compared with normal control gp. A significant increase in serum creatinine, uric acid and phosphorus levels with significant decrease serum calcium level were observed in groups (3 and 4). No significant changes ($P\leq0.05$) in other groups when compared with normal control group (Table 4).

**Histopathological findings:**

**Coccidial infected group (2) (Plate 1):** Examined sections form intestine exhibited developmental stages of Eimeria within most enterocytes with extravasated erythrocytes and lymphocytic aggregations within lamina propria and submuocsa Fig. (1, 2). As well as, liver showed perivascular area of coagulative necrosis which appeared as homogenous eosinophilic cytoplasm with loss of most nuclei beside presence of area of liquefactive necrosis which represented by empty spaces Fig. (3).While, kidney revealed interstitial round cells infiltrations between tubules and degenerative changes within renal tubular epithelium Fig. (4) (Table 5).

***C. perfringens* infected group (3) (Plate 2):** Intestinal sections revealed necrotic epithelial lining villi and desquamation most of them Fig. (5) beside presence of inflammatory cells infiltrations within lamina propria and submucosa. Moreover, liver revealed infiltrations of lymphocytes and heterophilic within some portal and periportal areas in addition to periportal necrotic area replaced by inflammatory cells infiltration mostly lymphocytes were also seen Fig. (6,7). Kidney exhibited focal interstitial area of round cells with congestion of renal blood vessels in addition to necrotic changes of some renal tubules Fig. (8) (Table 5).

**Coccidial and clostridia mixed infected group (4) (Plate 3):** Intestine showed denuded necrotic epithelium with presence of developmental stages of *Eimeria* within epithelial lining mucosa. Moreover, Congested blood vessels, extravasated erythrocytes and lymphocytic infiltration were also seen Fig. (9, 10). Liver showed necrotic area replaced by lymphocytic infiltrations. Dilated sinusoids with atrophied some hepatic cells were also detected Fig. (11). Kidney showed dilated renal blood vessels with degenerative changes of some tubules Fig. (12) (Table 5).

**All treated groups (5, 6, and 7) (Plate 4, 5 and 6):** Intestine showed apparently normal intestinal villi with preserved submucosal glands Fig. (13, 16 and 19). Liver showed apparently normal hepatic parenchyma in most parts. However, perivascular focal area of round cells were detected (Fig. 14).While, kidney showed normal glomerular corpuscles with some degenerative and necrotic changes of renal tubules Fig.(15) and some focal lymphocytic cells aggregations Fig. (18) (Table 5).

## DISCUSSION

Intestinal clostridia with coccidia infections are implicated in severe economic losses in poultry industry. *C. perfringens* isolated from NE suspected intestine were identified based on morphological, cultural and biochemical characteristics. Interestingly, in all total (55%) samples were found positive for *C. perfringens* from 100 diseased (NE suspected). The incidence *C. perfringens* in the current study had nearly coincided with the findings of **Abd El-Hamid *et al.*, (2015)** who recorded (65.1%) prevalence rates and **Asmaa *et al.*, (2017)** found that (55.9%) was positive for *C. perfringens*. While lower than obtained by **Ahmed and Abd El-Latif (2004)** that was (68.3%) and **Prerana *et al.*, (2018)** was (70%). And higher than those of **EL-Helw *et al.*, (2014)** and **Heidy *et al.*, (2015)** whom recorded prevalence rate 33.33%, and 45.9%, respectively. These differences may be due to the feed ingredients, feed additives and housing environment.

Concerning coccidia isolation it was represented by 39% and 34% of examined samples had mixed infection of *C. perfringens* and *Eimeria* spp. On the contrary another study found the detected *Eimeria* species, (325 chickens, 110 pigeons and 18 ducks) and absent in turkey and geese **(Nagwa *et al.*, 2013).**

With respect to the antimicrobial susceptibility testing of *C. perfringens* to ten different antimicrobial agents, *C. perfringens* isolates, indicated that, higher rates of sensitivity were observed to amoxicillin that was most effective against (81.8%) isolates of *C. perfringens* tested followed by cefotaxime (71 %) and amikacin (69%). These results nearly coincided with the findings of **Prerana *et al.*, (2018)** who also found that amoxicillin was most effective against (85.71%) of tested isolates. Other studies performed in the United States, China, and Norway had suggested that amoxicillin is the most effective against *C. perfringens* infection in poultry **(Lianco *et al.*, 2012**).

Furthermore, low and moderate resistance had been reported for lincomycin **(Lanckriet *et al.*, 2010)**, it supported our result that resistance to lincomycin was low 32.7%. However, other reports describe a greater number of lincomycin-resistant strains in turkey isolates **(Silva *et al.*, 2009).**

On the other hand, most of isolates were resistant to colistin (89%) followed by neomycin (72.7%). Similarly, **Gad *et al.*, (2011)** recorded highest resistance against neomycin and colistin. Moreover, **Prerana *et al.*, (2018)** found that 86.8% of isolates were resistant to colistin. Totally, most of isolates were resistant to at least 3 of the ten tested antimicrobial

agents, making them multidrug resistant (MDR). This finding was in agreement with that reported by **Osman and Elhariri (2013)**.The variation in the antimicrobial pattern might be due to indiscriminate use of these antibiotics as feed additive and prophylaxis as well as therapeutic agent in poultry industry.

C. *perfringens* is considered one of the normal commensals in humans and animals intestinal flora. Thus, differentiation between toxigenic and non-toxigenic strains is of significance. In this regards, toxigenic attributes of 13 isolates that demonstrated multidrug resistant phenotypes were studied by multiplex PCR and revealed that 7/13 (53.8%) of isolates were considered C. *perfringens* type A as were positive only for *cpa* gene encodes for alpha toxin. This result was consistent with the finding of other previous studies, as **Rachid *et al.*, (2017**) recorded that (100%) of their studied isolates belonged to *C. perfringens* type A. Moreover, **El - Helw *et al.*, (2014)** reported that all isolates obtained from intestine of turkey had toxin that belong to *C. perfringens* type A (33.33%), that in accordance with **Erol *et al.*, (2008**) they isolated and identified *C. perfringens* type A from turkey meat by multiplex PCR. The major typing toxins, type A strains produce only alpha toxin therefore, for a long time it was thought that alpha toxin was the major virulence factor in the pathogenesis of necrotic enteritis in poultry **(Van Immerseel *et al.*, 2009).**

Interestingly, our results revealed that, the experimentally infected turkey with coccidial group (2) recorded mortalities (40%) this result was in agreement with **Ashraf *et al.*, (2015)** who found that mortality rate 36%. Also infected group with *clostridium perfringens* (3) were resulted in mortality (30%) that matched with **McDevit *et al.*, (2006)** and **Aboubaker and Elbadawy (2017)** whom recorded mortality rate 36.67% and 40%, respectively when the birds challenged with 1.5 $\times 10^9$ cfu/ml. Furthermore, **Umar *et al.*, (2018)** recorded 30% mortality rate when the birds inoculated with *C. perfringens* $2.5\times 10^8$cfu/ml. The mortalities recorded in birds infected with *C. perfringens* may be due to the effect of its toxins **(Sameh *et al.*, 2005)**. On other side, other studies reported that there is no mortality detected during experiment **(Pedersen *et al.*, 2008)** who failed to induce disease and only a transient colonization with challenge strains had been obtained. Also, **Olkowanski *et al.*, (2006)** and **Malmarugan *et al.* (2010**) demonstrated that no clinical signs of NE and no mortality were recorded in an experiment involving infected with *C. perfringens*. **Vijay *et al.*, (2007)** attributed these differences in mortalities caused by infection with C. *perfringens* to many

stresses factor to which birds are exposed as wet litter, high temperature, ventilation management, crowdedness, type of ration and other management protocols.

With regard to performance indicators of amoxicillin treated group (6) we found that significant increase in body weight, weight gain and improvement in FCR throughout the experimental period post treatment (1313.33 ±12.02 , 396.67 ±6.01, 1.63 ±0.02 ), when compared with infected –non treated group (3) (1090 ±5.77, 280 ±2.89 , 1.84 ±0.03 ) may be due to antibacterial effect in suppression of *C. perfringens* and decreased its intestinal colonization (5 X $10^5$cfu/ml) which lead to prevention of necrotic enteritis as mentioned by **Watkins *et al.*, (1997)** these results were supported with **Lanckriet *et al.*, (2010)** and **Aboubaker and Elbadawy (2017)** whom stated that birds received amoxicillin at a dose of 20mg/kg body weight showed significant increase in body weight, weight gain and improvement in FCR.

On the other hand, administration of diclazuril in drinking water group (5) showed significant increase in body weight ,weight gain and improvement in FCR throughout the experimental period post treatment(1306.67 ±4.41 , 393.33 ±8.82 , 1.62 ±0.03 ) as compared with infected non treated group (2) (1101.67 ±7.26 , 310 ±15.27, 1.88 ±0.09 ), with decrease in coccidial oocysts count (4X$10^3$). These results agree with **El-Banna *et al.*, (2005)** and E**l-Dakhly *et al.*,(2006)** who reported that diclazural in the drinking water was the best choice for treatment of *Eimeria* spp.

Herein, higher mortalities 50% were recorded in group (4) with highest clostridia count (6X$10^9$) and highest coccidian count (6.4 X$10^4$). That explained the coccidial pathogens are the most predisposing factor of intestinal damage which result in releasing of intestinal protein into the lumen of intestinal tract this plasma provide necessary growth substrate for proliferation of clostridium **(Petit *et al.*, 1999).**

Of interest, the group (7) that treated with amoxicillin and diclazuril had the lowest intestinal colonization of *C. perfringens* (4X$10^5$) and decrease coccidial shedding (3.2X$10^3$) with reduced mortality rates. Similarly, another study found that *C. perfringens* count in intestine of birds post infection revealed a significant decrease in treated groups rather than control positive groups **(Soad *et al.*, 2015).** These results agree with those previously reported by **El-Banna *et al.* (2005) and El-Dakhly *et al.*, (2006)** who reported that diclazuril in the drinking water was used in the prevention and treatment of *Eimeria* infected chickens indicated by decrease the oocyst number and the lesion score in the treated groups than the

control positive.Regarding to the biochemical changes,hypoproteinemiaand hypoalbuminemia and decrease in the A/G ratio in all infected untreated groups may be due to anorexia, decrease feed intake, destructive effect of *C. perfringens* and its toxins on liver cells producing albumin **(Joan and Pannal, 1981)** and intestinal damage which resulted in releasing of intestinal protein into the lumen of intestinal tract **(Petit *et al.*, 1999).**

These results agree with **Aboubakr and Elbadawy (2017)** who found that broiler chickens infected with *C. perfringens* showed a significant reduction in total protein, albumin and A/G ratio post infection beside significant increase in globulin all over the experimental period post infection.

In this study the intestinal damage is confirmed by the histopathological observation which revealed necrotic epithelial lining villi and desquamation of most of them beside presence of inflammatory cells infiltrations within lamina propria and submucosa and it is in agreement with **Shojadoost *et al.*, (2012)** and **Soad *et al.*, (2015).** The explanation is that coccidian infection induced severe intestinal mucosal damage that permitted *C. perfringens* to induce necrotic enteritis **(Williams, 2002).**

Increase in total globulins, alpha1, alpha 2, beta 1and gamma globulins and decrease in the beta 2 globulin in groups (3 and 4) infected with *C. perfringens* may be due to bacterial infection which partially agree with **Aboubaker and Elbadawy (2017).** Group 2 showed significant increase in alpha 2 globulin and significant decrease in beta 2 globulin, the *Eimeria* spp. This partially parallel to that observed by **Augustine (1985)** who mentioned that, the alpha 2 and gamma -globulins were significantly increased in poults inoculated with either *E. adenoeides* or *E. meleagrimitis*; the alpha 1 and beta-globulins were unchanged. The changes in the total serum protein levels in *Eimeria*-infected turkeys appears to be due to the decrease in the albumin combined with increases in the alpha 2 and gamma-globulin fractions. El-evated globulin levels suggest liver or kidney disease **(Coles, 1997) and (Tully *et al.*, 2000)** or this usually is a result of sub-acute or chronic infections **(Coles, 1997**) who reported that, the gamma globulin is the immune globulin which increase in both acute and chronic infections.

No significant changes in treated groups (6 and 7) in serum total protein and albumin levels compared with the control groups indicate the effect of used drugs in the improvement of the case. This was evidenced by the histopathological finding of the intestine and liver of treated

groups and some increase in the A/G ratio due to the immune response to infection this agree with **Aboubaker and Elbadawy (2017)** who stated that amoxicillin displayed insignificant changes in total serum protein, albumin, globulin and A/G ratio all over the experimental period post administration when compared with negative control broiler chickens, and **Seham (1996)** who found that, healthy laboratory animals received amoxicillin showed non-significant changes in total protein, albumin and globulin.

The elevation in liver enzymes ALT, AST and ALP in *C. perfringens* infected groups (3 and 4) may be reflects the destructive effect of *C. perfringens* and their toxin on the liver tissue and agree with **Soad *et al.*, (2015)** and **Aboubakr and Elbadawy (2017).** This elevation in activity of liver enzymes may be due to pathological changes in liver after *C. perfringens* infections **(Coles, 1986)** which were necrotic area replaced by lymphocytic infiltrations and dilated sinusoids with atrophied some hepatic acini, or due to clostridia toxin that induced alterations in cellular permeability allows escape of liver enzymes into serum **(Joan and Panall, 1981).** Other infected and treated groups showed normal liver enzymes activity and it is confirmed by the microscopically as normal hepatic cells with preserved portal triads structures and stroma. These results were consistent with those reported by **Aboubakr and Elbadawy (2017)** and by **Bryan *et al.*, (1998)** who mentioned that, the improved liver enzymes post treatment in chicken infected by *C. perfringens* may be due to the antimicrobial effect of the used drugs in suppressing of microorganisms invade and retarding its metabolic activity. Increased triglycerides and VLDL levels and decrease in the HDL level in group 2 infected with coccidia may be due to anorexia and decrease feed intake, and increased triglycerides, cholesterol, LDL and VLDL levels with decrease in the HDL level in groups (3 and 4) infected with *C. perfringens* and mixed infection respectively may be related to the liver damage caused by *C. perfringens* and its toxins on liver cells and mobilization of body store during anorexia. These explanations were consonant with that reported by **Coles (1986)** and **Tully *et al.*, (2000**) who stated that cholesterol elevation is associated with liver disease and mobilization of body store during anorexia. Herein, the treatment by diclazuril and amoxicillin induce some improvement the lipograme parametrs in groups 5, 6 and 7 due to the antimicrobial effect of the used drugs in suppressing of microorganisms and prevent the liver destruction and these results confirmed histopathologically.

Regarding to kidney function tests there was hyperuricemia with significant increase in the creatinine level in addition to hypocalcaemia and hyperphosphatemia in the infected untreated

groups (3 and 4) reflects the renal disorder due to the infection with *C. perfringens*.

This agree with **Aboubakr and Elbadawy (2017)** who detected that experimental infection with *C. perfringens* in broiler chickens displayed a significant increase in uric acid and creatinine levels allover the experimental period post infection when compared with control broiler chickens. Our results confirmed histologically were kidney showed necrotic changes in renal tubules. Elevation in uric acid, creatinine in infected birds with *C. perfringens* could be attributed to the degenerative changes in kidney tubules preventing excretion of uric acid and creatinine increasing their levels in serum **(Kaneko, 1980).** Also, **Coles (1986) and Tully *et al.*, (2000)** declare that hyperurecimai, hypocalcaemia, hyperphophatemia and increase creatinine level is often associated with renal avian disease.The treated groups 5, 6 and 7 showed improvement in the kidney function which may be due to the effect of diclazuril and amoxicillin in treatment of the case and prevent the kidney destruction. These results resembling that obtained by **Aboubakr and Elbadawy (2017)** who reported that treatment of necrotic enteritis in broiler chickens induced no significant increase in serum creatinine and shift nearly toward the control levels which was confirmed histopathologicaly by normal renal tubules and glomerular structures.

## CONCLUSION

This work provides updated information on the characterization of toxigenic MDR *C. perfringens* and the incidence of *Eimeria* species in turkey. We concluded that amoxicillin and diclazuril in optimum doses would resolve most cases of coccidiosis and necrotic enteritis in turkey that had significant improvement in BW, BWG and lower FCR, in addition to reduction in mortalities, lowered number of oocysts, reduce the severities of necrotic enteritis (NE) and ameliorate the biochemical and pathological changes. It is advisable to periodically monitor the trends in resistance patterns of *C. perfringens* isolates, because this organism may be a source of resistance genes transferring to other species of bacteria, including animal and human pathogens.

**Table (3):** Effects of amoxicillin and diclazuril on body performance in healthy and experimentally infected turkey.

| Groups Age | | Control | Infected groups | | | Treated groups | | |
|---|---|---|---|---|---|---|---|---|
| | | | Coccidia (2) | Clost. (3) | Clost+ coccidia (4) | Coccidia (5) | Clost. (6) | Clost+ cocidia (7) |
| Body performance 21day-28day | BW(g) | 686.66 ±6.01$^{a}$ | 643.33 ±6.01$^{b}$ | 683.33 ±6.01$^{a}$ | 641 ± 6.35$^{b}$ | 643.33 ±6.01$^{b}$ | 683.33 ±6.01$^{a}$ | 641.66 ±10.1 $^{b}$ |
| | BWgain (g) | 231.67 ±3.33$^{a}$ | 216.67 ±4.40$^{b}$ | 230 ±2.88$^{a}$ | 215.67 ±2.33$^{b}$ | 216.66 ±4.40$^{b}$ | 230 ±2.88$^{a}$ | 218.33 ±6.01$^{b}$ |
| | F.C (g) | 299.33 ±3.33$^{a}$ | 296.33 ±2.90$^{a}$ | 302.66 ±5.77$^{a}$ | 296 ±2.08$^{a}$ | 298.66 ±3.1$^{a}$ | 296.66 ±1.66$^{a}$ | 293.33 ±3.66$^{a}$ |
| | FCR | 1.27 ±0.28$^{a}$ | 1.40 ±0.58 $^{a}$ | 1.28 ±0.2$^{a}$ | 1.37 ±0.24 $^{a}$ | 1.40 ±0.56$^{a}$ | 1.28 ± 0.51 $^{a}$ | 1.34 ±0.52$^{a}$ |
| Body performance at 28-35 days | BW(g) | 1021.67 ±1.67$^{a}$ | 788.33 ±6.01$^{d}$ | 914 ±3.78$^{c}$ | 731.67 ±6.01$^{e}$ | 911.67 ±4.41$^{b}$ | 916.67 ±6.01$^{b}$ | 910 ±5.77$^{b}$ |
| | BWgain (g) | 375 ±8.66$^{a}$ | 180 ±5.77$^{c}$ | 240 ±2.88$^{d}$ | 121.67 ±1.67$^{e}$ | 265 ±7.64$^{b}$ | 270 ±2.89$^{b}$ | 271.67 ±1.67$^{b}$ |
| | FCR | 1.41 ±0.01$^{d}$ | 1.78 ±0.06$^{b}$ | 1.57 ±0.44 $^{c}$ | 1.92 ±0.05$^{a}$ | 1.58 ±0.05$^{c}$ | 1.54 ±0.02$^{cd}$ | 1.57 ±0.01$^{c}$ |
| Body performance at 35- 42 day | BW(g) | 1531.67 ±6.01$^{a}$ | 1101.67 ±7.26$^{c}$ | 1090 ±5.77$^{c}$ | 965 ±2.89$^{d}$ | 1306.67 ±4.41$^{b}$ | 1313.33 ±12.02$^{b}$ | 1315 ±2.89$^{b}$ |
| | BWgain (g) | 511.67 ±6.01$^{a}$ | 310 ±15.27$^{c}$ | 280 ±2.89$^{d}$ | 231.67 ±6.01$^{e}$ | 393.33 ±8.82$^{b}$ | 396.67 ±6.01$^{b}$ | 403.33 ±8.82$^{b}$ |
| | FCR | 1.53 ±0.03$^{b}$ | 1.88 ±0.09$^{a}$ | 1.84 ±0.03$^{a}$ | 1.97 ±0.04$^{a}$ | 1.62 ±0.03$^{b}$ | 1.63 ±0.02$^{b}$ | 1.61 ±0.04$^{b}$ |

**-BW: body weight, FC: food consumption, FCR: food conversion rate.**

**-Values are expressed as mean ± standard error, n=3. Means within the same column carrying different superscripts are significant at ($P \leq 0.05$)**

**Table (4):** Some biochemical changes of tested groups (Mean±SE) (n=5).

| Groups Parameters | Control (1) | Infected groups | | | Treated groups | | |
|---|---|---|---|---|---|---|---|
| | | Coccidia (2) | Clost. (3) | Clost. +coccidia (4) | Coccidia (5) | Clost. (6) | Clost +cocidia (7) |
| Total protein (g/dl) | 4.04 ±0.02$^{a}$ | 3.51 ±0.01$^{c}$ | 3.71 ±0.03$^{b}$ | 3.81 ±0.02$^{b}$ | 3.71 ±0.01$^{b}$ | 3.89 ±0.23$^{ab}$ | 3.98 ±0.04$^{ab}$ |
| Albumin (g/dl) | 2.67 ±0.12$^{a}$ | 1.96 ±0.05$^{c}$ | 1.93 ±0.01$^{c}$ | 1.8 ±0.03$^{c}$ | 2.2 ±0.05$^{b}$ | 2.31 ±0.03$^{ab}$ | 2.55 ±0.03$^{a}$ |
| Globulin (g/dl) | 1.39 ±0.11$^{cd}$ | 1.46 ±0.1$^{c}$ | 1.78 ±0.04$^{b}$ | 2.01 ±0.04$^{a}$ | 1.5 ±0.06$^{c}$ | 1.58 ±0.01$^{c}$ | 1.42 ±0.08$^{c}$ |
| A/G ratio | 1.92 ±0.25$^{a}$ | 1.34 ±0.06$^{c}$ | 1.08 ±0.03$^{d}$ | 0.9 ±0.03$^{d}$ | 1.47 ±0.09$^{b}$ | 1.46 ±0.05$^{b}$ | 1.80 ±0.12$^{ab}$ |
| Alpha 1 globulin (g/dl) | 0.27 ±0.04$^{c}$ | 0.33 ±0.01$^{bc}$ | 0.39 ±0.01$^{ab}$ | 0.44 ±0.06$^{a}$ | 0.31 ±0.02$^{c}$ | 0.31 ±0.01$^{c}$ | 0.29 ±0.02$^{c}$ |
| Alpha 2 globulin (g/dl) | 0.26 ±0.02$^{e}$ | 0.44 ±0.01$^{c}$ | 0.49 ±0.01$^{b}$ | 0.55 ±0.01$^{a}$ | 0.42 ±0.01$^{cd}$ | 0.35 ±0.01$^{d}$ | 0.38 0.02$^{d}$ |
| Beta 1 globulin (g/dl) | 0.20 ±0.02$^{c}$ | 0.25 ±0.01$^{bc}$ | 0.29 ±0.01$^{ab}$ | 0.33 ±0.01$^{a}$ | 0.24 ±0.01$^{bc}$ | 0.25 ±0.01$^{bc}$ | 0.21 0.01$^{c}$ |
| Beta 2 globulin (g/dl) | 0.27 ±0.02$^{a}$ | 0.15 ±0.003$^{cd}$ | 0.16 ±0.01$^{cd}$ | 0.18 ±0.003$^{cd}$ | 0.16 ±0.01$^{cd}$ | 0.23 ±0.01$^{b}$ | 0.20 ±0.01$^{bc}$ |
| Gamma globulin (g/dl) | 0.38 ±0.01$^{cd}$ | 0.39 ±0.01$^{bc}$ | 0.44 ±0.01$^{b}$ | 0.51 ±0.02$^{a}$ | 0.36 ±0.023$^{cd}$ | 0.33 ±0.03$^{cd}$ | 0.32 ±0.01$^{d}$ |
| ALT(U/L) | 9.97 ±1.24$^{c}$ | 10.18 ±1.00$^{c}$ | 24.01 ±0.63$^{a}$ | 27.59 ±1.19$^{a}$ | 10.33 ±1.24$^{c}$ | 11.89 ±1.01$^{c}$ | 10.91 ±1.18$^{c}$ |
| AST(U/L) | 32.7 ±4.73$^{c}$ | 21.95 ±3.59$^{c}$ | 222.33 ±6.17$^{a}$ | 200.5 ±1.04$^{b}$ | 38.67 ±2.45$^{c}$ | 19.89 ±1.65$^{c}$ | 36 ±1.52$^{c}$ |
| ALP(U/L) | 301.43 ±6.41$^{c}$ | 309.3 ±11.61$^{c}$ | 397.5 ±4.7$^{b}$ | 702.97 ±6.94$^{a}$ | 324.766 ±12.19$^{c}$ | 321.67 ±12.04$^{c}$ | 347.7 ±14.74$^{c}$ |
| TG (mg/dl) | 106.8 ±1.56$^{d}$ | 126.53 ±0.5$^{c}$ | 175.97 ±5.13$^{a}$ | 146.23 ±0.5$^{b}$ | 119.57 ±1.31$^{cd}$ | 113.63 ±9.44$^{d}$ | 111.77 ±4.33$^{d}$ |
| Colesterol (mg/dl) | 149.6 ±0.5$^{c}$ | 169.2 ±2.48$^{c}$ | 235.67 ±4.78$^{a}$ | 204.9 ±1.21$^{b}$ | 170.16 ±2.45$^{bc}$ | 138.83 ±2.31$^{cd}$ | 172.96 8.56$^{bc}$ |
| HDLP (mg/dl) | 54.13 ±0.61$^{a}$ | 49.00 ±0.58$^{b}$ | 47.97 ±0.11$^{b}$ | 49.35 ±0.44$^{b}$ | 52.41 ±1.24$^{a}$ | 46.48 ±1.71$^{b}$ | 56.91 ±0.13$^{a}$ |
| LDL (mg/dl) | 74.24 ±0.16$^{c}$ | 94.89 ±4.27$^{bc}$ | 152.5 ±5.68$^{a}$ | 126.36 ±1.64$^{ab}$ | 93.50 ±4.01$^{bc}$ | 71.29 ±1.28$^{c}$ | 93.70 ±8.81$^{bc}$ |
| VLDL (mg/dl) | 21.23 ±0.33$^{c}$ | 25.31 ±1.22$^{b}$ | 25.19 ±1.03$^{b}$ | 29.18 ±0.13$^{a}$ | 24.25 ±0.45$^{b}$ | 21.06 ±1.85$^{c}$ | 22.35 ±0.87$^{c}$ |
| Createnine (mg/dl) | 0.34 ±0.01$^{c}$ | 0.30 ±0.01$^{c}$ | 0.41 ±0.03$^{b}$ | 0.48 ±0.01$^{a}$ | 0.31 ±0.02$^{c}$ | 0.33 ±0.02$^{c}$ | 0.36 ±0.02$^{c}$ |
| Uric acid (mg/dl) | 7.17 ±0.41$^{c}$ | 8.13 ±0.09$^{bc}$ | 8.48 ±0.47$^{ab}$ | 9.21 ±0.37$^{a}$ | 8.04 ±0.08$^{bc}$ | 7.72 ±0.17$^{bc}$ | 7.9 ±0.19$^{bc}$ |
| Calcium (mg/dl) | 9.38 ±0.25$^{a}$ | 8.05 ±0.30$^{b}$ | 7.52 ±0.16$^{cd}$ | 7.62 ±0.08$^{cd}$ | 8.96 ±0.09$^{ab}$ | 9.00 ±0.09$^{ab}$ | 9.08 ±0.15$^{ab}$ |
| Inorganic phosphrus (mg/dl) | 2.91 ±0.08$^{b}$ | 3.11 ±0.16$^{b}$ | 4.00 ±0.08$^{a}$ | 3.99 ±0.05$^{a}$ | 2.98 ±0.07$^{b}$ | 3.13 ±0.07$^{b}$ | 2.99 ±0.03$^{b}$ |

**n: number of samples A/G: Albumin globulin ratio TG: Triglycerides HDL: High density lipoprotien LDL: low density lipoprotien VLDL: Very low density lipoprotien * Significant at P≤0.05**

**Table (5):** Summarized the main histopathological lesions scores among different groups.

| Groups Organs | Lesions | Infected groups | | | Treated groups | | |
|---|---|---|---|---|---|---|---|
| | | Coccidia (2) | Clost (3) | Clost+coccidia (4) | Coccidia (5) | Clost (6) | Clost+cocidia (7) |
| Intestine | Necrotic enterocytes | ++ | +++ | +++ | - | - | - |
| | Lymphocytic infiltration | ++ | + | ++ | + | - | - |
| | Dilated blood vessels | ++ | ++ | ++ | - | - | - |
| | Hemorrhage | +++ | + | ++ | - | - | - |
| Liver | Degenerative changes | + | ++ | ++ | + | - | + |
| | Necrotic areas | ++ | +++ | ++ | - | - | - |
| | Lymphocytic infiltrations | ++ | + | ++ | + | + | + |
| | Heterophilic infiltrations | - | + | ++ | - | + | - |
| | Congested blood vessels | + | ++ | ++ | + | - | - |
| Kidney | Degenerative changes | ++ | ++ | ++ | + | + | - |
| | Necrotic changes | + | + | ++ | + | + | - |
| | Lymphocytic infiltrations | ++ | ++ | ++ | + | + | - |
| | Congestion of renal blood vessels | + | ++ | +++ | - | - | - |

**- = No altreations + = Mild (25-35% altreations) ++ = Moderate (40-65% altreations) +++ = Severe (up to 65% altreations)**

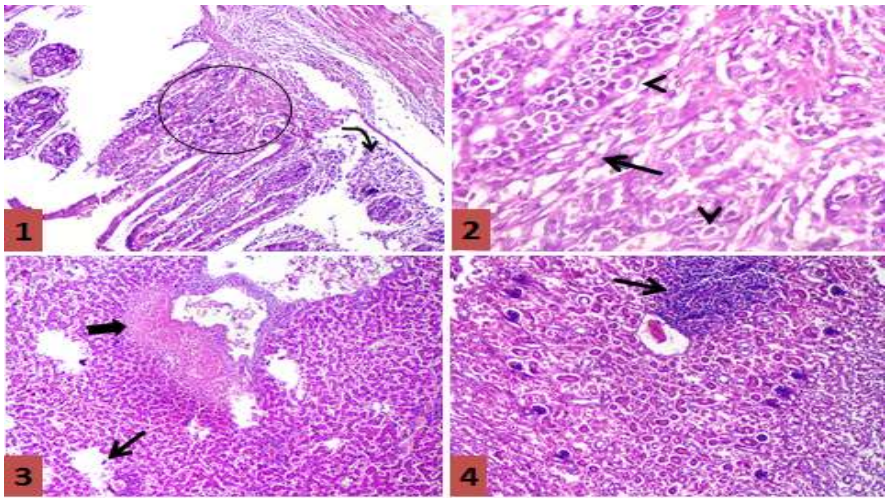


**Plate 1:** Photomicrographs of group (2) infected with Eimeria showing:

**Fig. (1, 2)**: Intestine with developmental stages of Eimeria within most enterocytes **(arrow head)** with extravasated erythrocytes **(arrow)** and lymphocytic aggregations within lamina propria and submuocsa **(curved arrow) (H&E (1) X100, (2) X400).**

**Fig. (3):** Liver with perivascular area of coagulative necrosis **(thick arrow) (arrow)**. **(H&E X100).**

**Fig. (4):** Kidney with focal round cells infiltrations **(arrow). (H&E X100).**

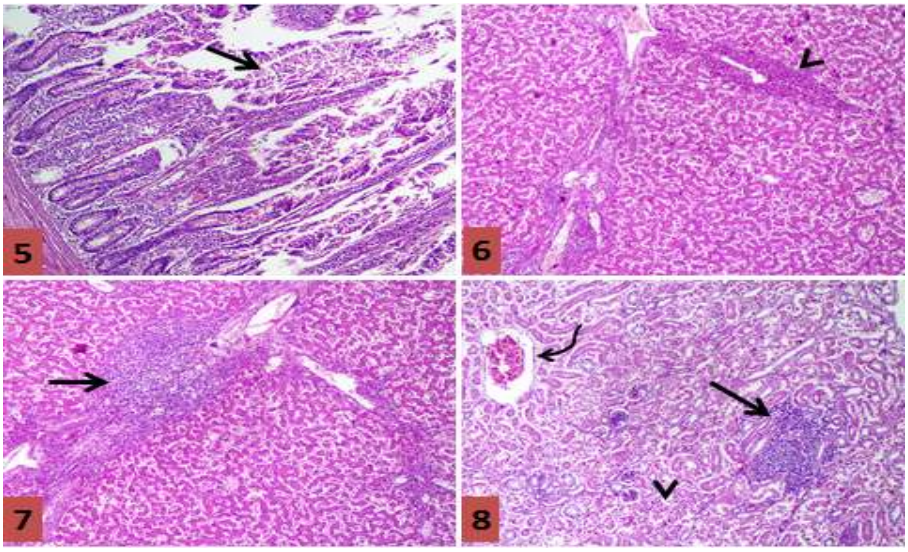


**Plate (2):** Photomicrographs of group (3) infected with clostridia showing:

**Fig. (5):** Intestine with necrotic epithelial lining villi and desquamation most of them **(arrow)**. **(H&E X100)**

**Fig. (6, 7):** Liver with lymphocytes and heterophiles infiltrations within portal areas **(arrow head)** with necrotic area replaced by lymphocytes **(arrow)**. **(H&E X100).**

**Fig. (8):** Kidney with focal area of round cells **(arrow)** with congestion of renal blood vessels **(curved arrow)**. **(H&E X100).**

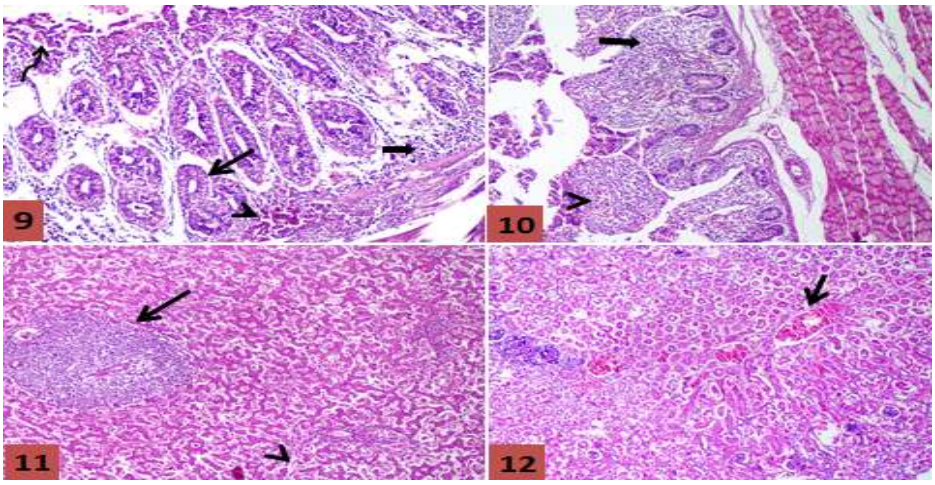


**Plate (3):** Photomicrographs of mixed infection with coccida and clostridia (group 4) showing:

**Fig. (9, 10):** Intestine with denuded necrotic epithelium **(curved arrow)** with presence of developmental stages of Eimeria **(arrow)**, congested blood vessels, extravasated erythrocytes **(arrow head)** and lymphocytic infiltration **(thick arrow)**. **(H&E X400).**

**Fig. (11):** Liver with necrotic area replaced by lymphocytic infiltrations **(arrow)** beside dilated sinusoids with some atrophied hepatic cells **(arrow head). (H&E X100).**

**Fig. (12):** Kidney with congested renal blood vessels **(arrow). (H&E X100).**

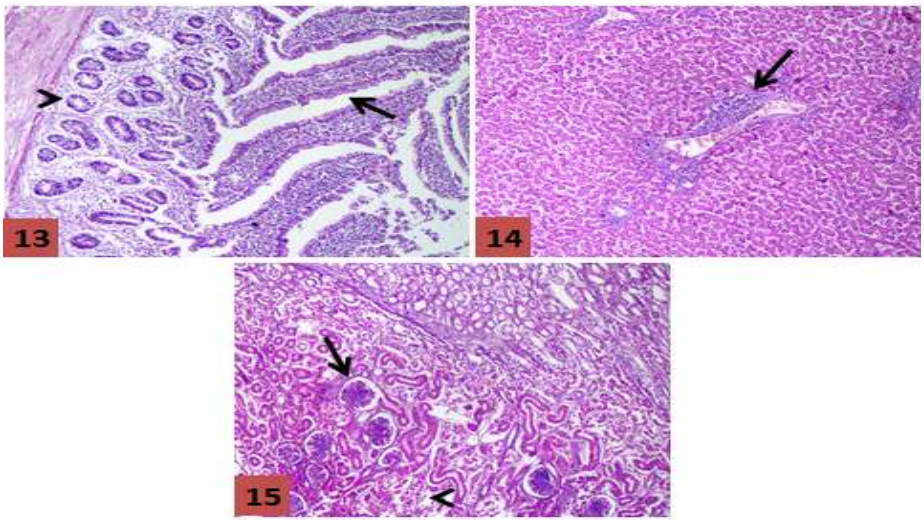


**Plate (4):** Photomicrographs of treated coccida infection with diclazuril group (5) showing:

**Fig. (13):** Intestine with apparently normal intestinal villi **(arrow)** with intact submucosal glands **(arrow head)**. **(H&E X 200).**

**Fig. (14):** Liver with perivascular focal area of round cells **(arrow). (H&E X100).**

**Fig. (15):** Kidney with normal glomerular corpuscles **(arrow)** with mild degenerative changes of renal tubules **(arrow head). (H&E X100).**

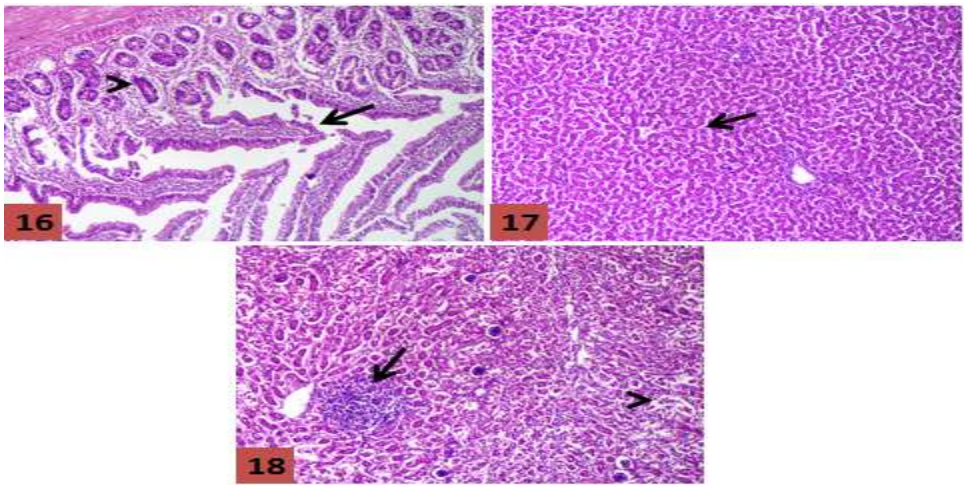


**Plate (5):** Photomicrographs of treated clostridia infection with amoxicillin group (6) showing:

**Fig. (16):** Intestine with normal mucosal layer with villus epithelium **(arrow)**, lamina propria, submucosa with glands **(arrow head)** and muscular layer**. (H&E X100).**

**Fig. (17):** Liver with intact hepatic cells **(arrow)** and portal triads. **(H&E X100).**

**Fig.(18):** Kidney with focal lymphocytic cells aggregations between renal tubules and glomerular structures **(arrow)** beside some necrotic renal tubules **(arrow head). (H&E X100).**

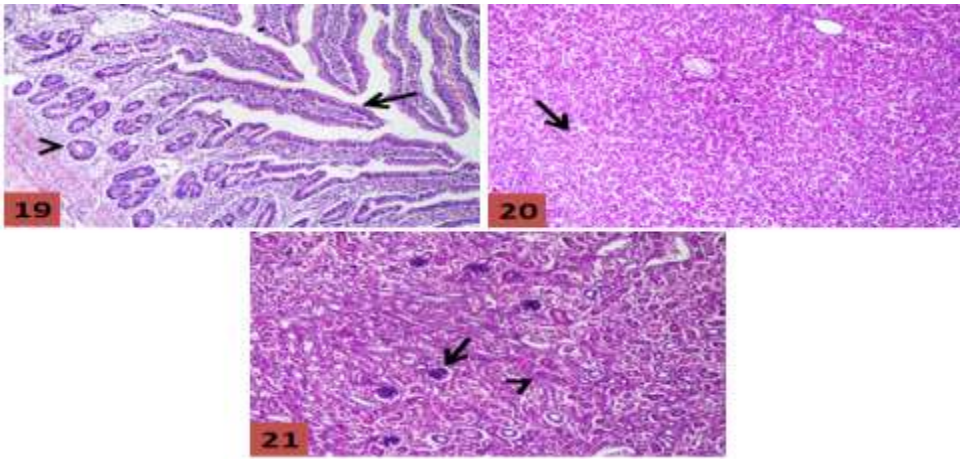


**Plate (6):**Photomicrographs of treated mixed infection of coccidia and clostridia with amoxicillin and diclazuril group (7) showing:

**Fig. (19):** Intestine with apparently normal intestinal villi **(arrow)**, submucosal glands **(arrow head)**, musculosa and serosal structures. **(H&E X400).**

**Fig.(20):** Liver with some degenerative changes within hepatocytes as vacuolar degenerations **(arrow)**. **(H&E X100).**

**Fig. (21):** Kidney with normal renal tubules **(arrow head)** and glomerular structures **(arrow)**. **(H&E X100).**

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# تقييم التغيرات المرضيه للعدوى المشتركه بين الكوكسيديا والتهاب الامعاء الناخر فى الرومى

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## الملخص العربى

يعتبر الالتهاب المعوي التنكرزي من اهم المشاكل فى صناعه الدواجن عالميا. بالرغم من ذلك يوجد القليل من المعلومات عن تطورات المرض فى قطعان الرومى بعد الفحص البكتريولوجى والطفيلى كانت 55% و 39% إيجابية لكلا من الكلوستريديم بيرفرينجيز والكوكسيديا على التوالى و34% كانت مختلطه بعدوى الكلوستريديم بيرفرينجيز و الكوكسيديا. و بفحص الانواع القادره على انتاج السموم بين المعزولات باستخدام انزيم البلمره المتعدد اظهرت النتائج ان عترات الكلوستريديم بيرفرينجيزتتنمى للنوع (أ). كما اظهرت نتائج الحساسيه ان اعلى نسبه مقاومه ضد الكولستين 89% يليه النيوميسين 72.7% و كان الاموكسيسيلين الكثر فاعليه بنسبه 81.8% ثم السيفوتاكسيم بنسبه 71%. لذلك تهدف هذه الدراسه الى عمل عدوى اصطناعيه للالتهاب المعوى التنكرزى مع العدوى بالكوكسيديا لتحديد التغيرات على اداء النمو و التغيرات الكيميائيه فى السيروم بالاضافه الى التغيرات النسيجيه. ولاجراء هذه التجربه استخدمنا 70 كتكوت رومى، تم تقسيمهم عشوائيا الى 7 مجموعات متساويه. المجموعه الاولى تركت كمجموعه ضابطه سلبيه، الثانيه تم اصابتها تجريبيا بالكوكسيديا، الثالثه تم اصابتها تجربيا بالكلوستردیا و الرابعه تم عمل عدوى مختلطه بالكوسيديا والكلوستردیا اما المجموعه الخامسه والسادسه والسابعه فتم علاجهم بالديكلازوريا والاموكسيسيلين والاثنين معا على التوالى بعد اجراء عدوى صناعيه لهم بالكوكسيديا والكلوستردیا والاثنين معا على التوالى.وباجراء عدوى اصطناعيه بكلا من كلوستيريديم بيرفرييجينز نوع (أ) والكوكسيديا ادى الى تقليل الاوزان وزياده فى معدلات التحول الغذائى ونقص فى معدلات النمو بالاضافه الى حدوث تلف فى انسجه الامعاء والكبد والكلى ادى الى خلل فى وظائف الكبد والكلى ونقص فى البروتين الكلى والنوعى وخلل فى مستويات الدهون الكليه والنوعيه والذى اظهرته التغيرات الباثولوجيه والباثولوجيه الاكلينيكيه. ووجد ان الاموكسيسيلين والديكلوسول لهما تاثير كبير و فعال فى زياده الاوزان وتحسين معدلات النمو وانخفاض معدلات التحول الغذائى وتقليل نسبه النفوق وتقليل عدد حويصلات الكوكسيديا و العد البكتيرى لميكروب الكلوستريديا وايضا تحسين التغيرات الباثولوجيه والباثولوجيه الاكلينيكيه.