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Research Article

The Effect of Cephardine on Clinicopathological Pictures of Experimental *Salmonella Enteritidis* and *E. coli* Infections in Broiler Chickens

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ABSTRACT

The objective of this study is to determine the effect of cepharidine on clinical and pathological pictures, of *E. coli* infection (colisepticaemia) in broiler chickens and *S. Eteritidis* infection. Three weeky-old meat type chicks (n=100) allotted into 5 equal groups (1-5) with 2 replicates of 10 each were used in this study. Duration of the experiment extended from one day of age up to slaughter (42 days). The birds fed mash diet for 3 phases: starter (1-14 days), grower (15-28 days) and finisher (29-35 days). For experimental induction of colisepticaemia; experimented chickens of groups 1and 2 were subcutaneously infected with 4.5×10^6 CFU/ml/bird of *E. coli* serogroup O1in PBS. Birds of group 3 and 4 were oraly inoculated with *Salmonella Enteritidis* in a dose of 4×10^5 CFU/ml/bird. Those of group 5 were kept without infection as negative control group. Cepharidine supplementation to E. coli and Salmonella infected broiler chickens improved reduced clinical, both gross and histopathological lesion scores. Conclusively; Ceharidine treatment could play a positive role in controlling of colisepticaemia and S.Enteritidis infection in Broiler Chickens.

Key words: Cephardine, *Salmonella Enteritidis*, *E. coli*, Broiler Chickens

INTRODUCTION

Avian colibacillosis and salmonellosis are still constituting a threat to poultry industry all over the world, not only because they are associated with severe economic losses, but they induce different disease conditions as well. In addition, both diseases are of zoonotic importance as they are communicable to human and may cause food borne illnesses in different countries (Helmy *et al*., 2017).

Avian colibacillosis is considered as one of the main causes of mortality and morbidity among poultry farms. In poultry intestine, normal microflora *E. coli* is present as a normal inhabitant but highly Pathogenic Escherichia coli (APEC) has the ability to spreads into several internal organs inducing systemic septicemia associated with fatal infection (Oh *et al.,* 2011). Septicemia associated with death are the main characteristic symptoms that are associated with acute infection, however in subacute forms several disease conditions could be observed such as enteritis, omphalitis, perihepatitis, pericarditis, and air-saculitis (Gast, 2013).It was estimated that pathogenic Escherichia coli (APEC) constitute about 10-15% of intestinal coliform bacteria (Nolan *et al*., 2013).

A variety of *Salmonella* species can induce avian salmonellosis that cause huge economic losses in poultry field as a result of decrease production and high mortality among infected birds (Haider *et al*., 2004, Islam *et al*., 2006).

Beside their public health significance; motile salmonellae are principally of concern as a cause of food born disease in human and are major portion of human salmonellosis. The main salmonella serovars involved in human food poisoning outbreaks were *S. typhimurium*, *S. hadar*, *S. Eneritidis and S. agona*. A major portion of illnesses were associated with meat and poultry (30.6% of incidents and 42.7% of cases) (Todd, 1989). In addition to *S. gallinarum-pullorum*, serogroup D1 include *S. enteritidis*, *S. panama* and *S. dublin* (Kabir, 2010). Other serotypes such as *Salmonella* Enteritidis and *Salmonella* Typhimurium which are known as paratyphoid (Gast, 2013). The prevalence rate of salmonella infection reached 53.25% in adult layers, 14.55% in brooding, and 16.10% in growing and pullets (Rahman *et al*., 2004).

As infections of domestic poultry with salmonellae are expensive both for the poultry industry and for society as a whole (Gast, 2013), existing a program by using antibiotics with the aim to reduce paratyphoid infections from broiler flocks and to reduce the significance of animal food as a source of infection to man cannot be ignored. Until now, using antibacterials therapy is the main significant tool in poultry farms in combating colibacillosis and salmonellosis infections.

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Cephalosporins antibacterial group acts by inhibition of bacterial cell wall synthesis and are typically more resistant to deactivation by β-lactamases (Webster, 2001). Cehpradine is a first generation cephalosporin antibiotic. In poultry, it is used against many microorganisms including cocci (other than enterococcus) and gram-positive bacilli and some gram-negative bacilli. Clinical studies in animals indicated that cephardine is affective against infectious conditions caused by a broad spectrum of gram negative and gram-positive organisms when given orally (Gadebush *et al.,* 1972). Moreover, it has been shown to have a lower order of oral toxicity and nontoxic effect after parenteral administration (Hassert, 1973).

Recently, the bioavailability, pharmacokinetics and tissue residues of cephradine in healthy and infected broiler chickens infected experimentally with *Salmonella entretidis* (EL-Sayed *et al*., 2016) and with *E.coli*. (M. Aboubakr and M. Elbadawy, 2017) were studied**.** However, the clinical effect of cephradine on the clinical and pathological pictures of these infections were not investigated in these studies. Therefore; the main objective of our study is to determine the possible effectiveness of cephardine on the clinicopathological pictures of experimental infection with *E. coli* or *Salmonella Enteritidis* (*S. Enteritidis*) as potent chicken enteropathogens.

MATERIALS AND METHODS

Experimental birds

A total of 100; one-day-old meat type chicks obtained from a commercial hatchery were used. The chicks were allotted into 5 groups (1-5) by 2 replicates consisting of 10 each. All birds were housed in separate rooms at a density of 10 birds / m2 and supplied with commercial broiler ration *ad libitum*. Birds were vaccinated against Newcastle disease at 5 and at 18 days via oral route in drinking water and against infectious bursal disease vaccine at 14 days of age via intraocular according to vaccination program in Egyptian field.

Bacterial strains and Experimental infection

E. coli: *E. coli* serogroup O1strain obtained from Animal Health Research Institute was used as a challenge organism. It was identified after (Cruickshank *et al*., 1970). The organism was inoculated on a slope agar and incubated for 24 hours at 37°C. Colonies were collected and suspended in saline to a density of McFarland No.4 standard for use. Infection was given S/C in a dose of 4.5 $x10^6$ CFU/ bird at 21 days of age.

Salmonella Enteritidis: *S. Enteritidis* strain obtained from Animal Health Research Institute was used as a challenge organism. Infection was given orally with 4 X 10^5 CFU / bird at 21 days of age after (Awaad *et al.*, 2003).

Medication

Cephradine is a first generation cephalosporin antibiotic. It is a water-soluble powder. Each 100 gm of powder was containing 20 gm cephradine base.

Experimental design

At 3 weeks of age, chickens of group 1 and 2 were inoculated with *E. coli* serogroup O1 while chickens in group 3 and 4 were inoculated with *S. Enteritidis*. Chickens of group 5 kept without infection and served as a negative control group. Birds in group 1 and 3 were treated with cephardine in a dose of (20 mg/kg B.Wt.) in drinking water twice daily for 5 successive days, once appearance of first clinical signs of the infections. All birds were kept for 3 weeks observation period for clinical signs and mortality. Dead as well as sacrificed survived birds at end of observation period (42 days of age) were subjected to postmortem lesion scoring. Specimens from heart, liver and spleen were collected for histopathological examination and microscopic lesion scoring.

Samples collection and histopathological examination

Specimens from heart, liver and spleen were collected at the end of experiment and fixed in 15% buffered formalin. Samples were prepared and stained according to methods described by (Bancroft *et al*., 1996) and scored for histopathological lesions according to the method described by (Rosales *et al*., 1989).

RESULTS AND DISCUSSION

In the present study, the observed clinical signs manifested were depression, ruffled feathers, increased thirst, deprived appetite and huddling together beside diarrhea, pasty vent and lameness that observed 24 hours post infection in some of the infected birds. These symptoms are similar to those reported earlier by Oh *et al.* (2011) and Gast *et al.* (2013) for *E. coli* and by (Shivaprasad 2000); Shivaprasad and Borrow (2013) for Salmonellae.

Cephardine treatment reduced the severity of clinical signs and the mortality rate in *E. coli* infected birds from 25% (5/20) to 15% (3/20) and the mortality in *Salmonella Enteritidis* infected group from 15% (3/20) to 5% (1/20) (Table 1, Fig. 1). It was estimated during the first week of age the mortality may reach up to 50% of total poultry loses (Yassin *et al.,* 2009) and bacterial infection, primarily *E. coli* infections are responsible for over 50% of these mortalities (Olsen *et al.,* 2012). Kemmett *et al.,* (2017) reported that the early mortality of commercial broiler chickens could be contributed significantly to systemic *E. coli* infections.

Post mortem examination of dead as well as sacrificed survived chickens at the end of observation period showed septicemic picture manifested as congestion of paranchymatous organs (heart, liver, spleen and kidneys) with petecheal or ecchymotic haemorrhages in these organs together with unabsorbed inflamed yok sacs with various degrees of pericarditis, perihepatitis and air-saculitis specially in *E. coli* infected groups. Generally speaking, the lesions were more prominent in untreated groups as compared with cepharidine treated ones. The total lesion scores reached 12 and 15 in *S. Enteritidis* infected treated and untreated groups respectively, while lesion scores reached 12 and 19 in *E. coli* infected treated and untreated groups respectively (Table 1, Fig b). These findings indicate that cephradine provided a beneficial effect for treatment of *Salmonella entretidis or E. coli* infections. It is already documented that cephalosporins antibacterial group acts by inhibition of the bacterial cell wall synthesis and are typically more resistant to deactivation by β-lactamases (Webster, 2001). Additionally, the activity of cephradine against some entero-bacteria, including strains

Table 1: Mortality and pathological lesion score of broiler chickens experimentally infected with *Salmonella Enteritidis or E. Coli* and treated with Cephrdine.

Case No.	*Salmonella Enteritidis*		*E. Coli*		*Blank Control*
	Cephrdine treated	Non-treated	Cephrdine treated	Non-treated	
1	++++	-	-	+	-
2	++	-	++	-	-
3	-	-	++	+	-
4	-	-	+	++	-
5	-	++	+	-	-
6	-	+	-	++	-
7	-	-	-	-	-
8	-	++	++	+	-
9	+	++	-	++	-
10	+	+	-	++++	-
11	-	+	-	-	+
12	-	+	-	++++	-
13	+	++	-	-	-
14	-	-	++	-	-
15	-	++	+	++	-
16	+	+	+	D	-
17	-	-	-	D	-
18	+	D	D	D	+
19	++	D	D	D	-
20	D	D	D	D	-
Total score	12	15	12	19	2

D= Dead bird; +=Positive; -=Negative).

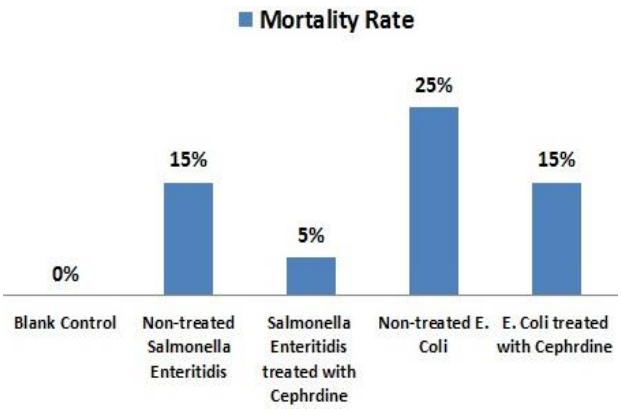

Fig. 1: Mortality rate of broiler chickens experimentally infected by *Salmonella Enteritidis or E. Coli* and treated with Cephrdine.

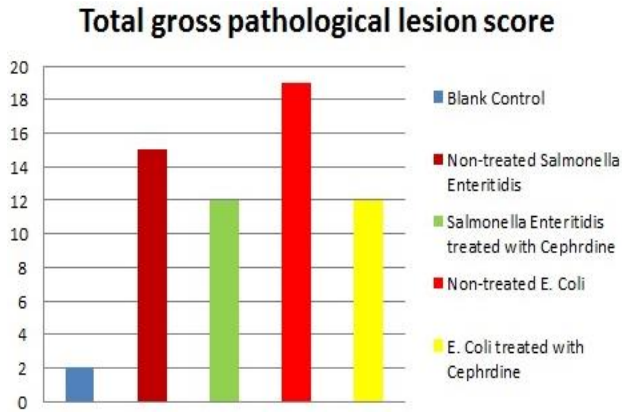

Fig. 2: Pathological lesion scores of broiler chickens experimentally infected by *Salmonella Enteritidis or E. Coli* and treated with Cephrdine.

of *E coli, Shegella spp., proteus mirabilis, Klebsiella pneumonia and Salmonellae* was also reported earlier by Martindale (1993). Our findings support the earlier studies conducted by EL-Sayed *et al*. (2016) and M. Aboubakr and M. Elbadawy (2017) who concluded that the oral bioavailability of cephradine is excellent and is recommended to be used against *Salmonella Entretidis or* colisepticemic infections in broilers chickens.

The histopathological findings were recorded in liver, heart and spleen of examined birds and revealed the following lesions:

Liver

Microscopically, examined sections from chicken infected with *E. coli* revealed marked diffuse hepatitis manifested by diffuse inflammatory cells infiltration mainly heterophils throughout the hepatic parenchyma as well as impaction of the hepatic sinusoids with heterophils (Fig. 1). Vacuolar degeneration of hepatocytes, dissociation of hepatic plates, focal hepatocellular necrosis associated with inflammatory cells infiltration (Fig. 2) and focal hepatic hemorrhage were also recorded in all examined sections. Moreover, congestion of hepatoportal blood vessels, portal infiltration with leucocytes, dilatation of bile duct with marked necrosis and desquamation of its epithelial lining associated with pericholangiolar fibrosis were observed (Fig. 3). Additionally, all hepatic sections infected with *E. coli* revealed perihepatitis, vacuolization and necrosis of subcapsular hepatocyte (Fig. 4). Conversely, liver of *E. coli* infected and treated chicken showed improvement in the previously mentioned alterations as examined sections revealed Kupffer cells activation and portal infiltration with heterophiles (Fig. 5). Regarding liver of chicken infected with *Salmonella enteritidis*, examined sections revealed dilatation and congestion of hepatic sinusoids, fatty change of hepatocytes (Fig. 6), sinusoidal leucocytosis and focal hepatic necrosis completely replaced by leucocytic cells aggregation (Fig. 7). On the other hand, some examined

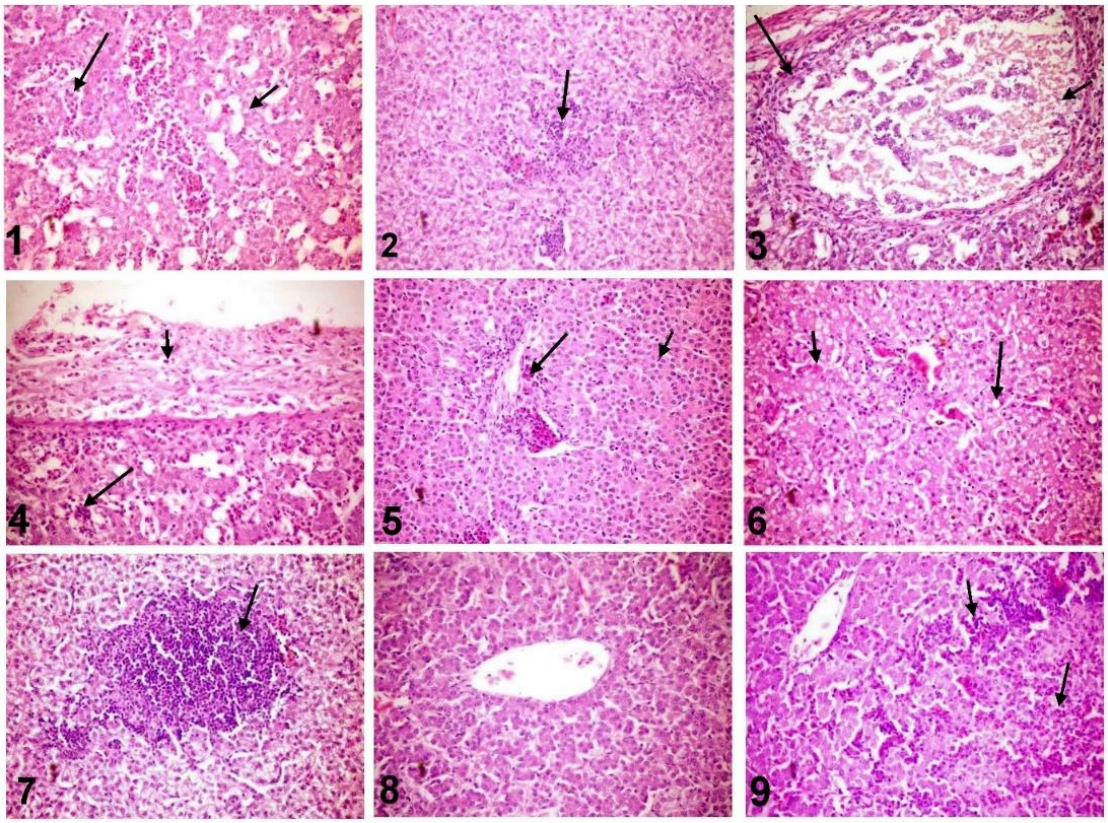

Plate 1: Liver of chickens infected with *E. coli* (Figs1-5) or *Salmonella Enteritidis* (Figs. 6-9), (H & E stain X 200): Fig. (1): showing marked diffuse hepatitis described by hepatocellular degeneration (short arrow) and diffuse inflammatory cells infiltration mainly heterophils throughout the hepatic parenchyma (long arrow). Fig. (2): showing focal hepatocellular necrosis associated with inflammatory cells infiltration (arrow). Fig. (3): showing dilatation of bile duct with marked necrosis and desquamation of its epithelial lining (short arrow) associated with pericholangiolar fibrosis (long arrow). Fig. (4): showing Perihepatitis (short arrow), vacuolization and necrosis of subcapsular hepatocyte (long arrow). Fig. (5): treated with cephardine showing Kupffer cells activation and portal infiltration with heterophiles Fig. (6): showing dilatation and congestion of hepatic sinusoids, fatty change of hepatocytes. Fig. (7): showing focal hepatic necrosis completely replaced by leucocytic cells aggregation (arrow). Fig. (8): treated with cephardine showing no histopathological changes. Fig. (9): treated with cephardine showing focal hepatic necrosis (short arrow) associated with heterophilic cells infiltration (long arrow).

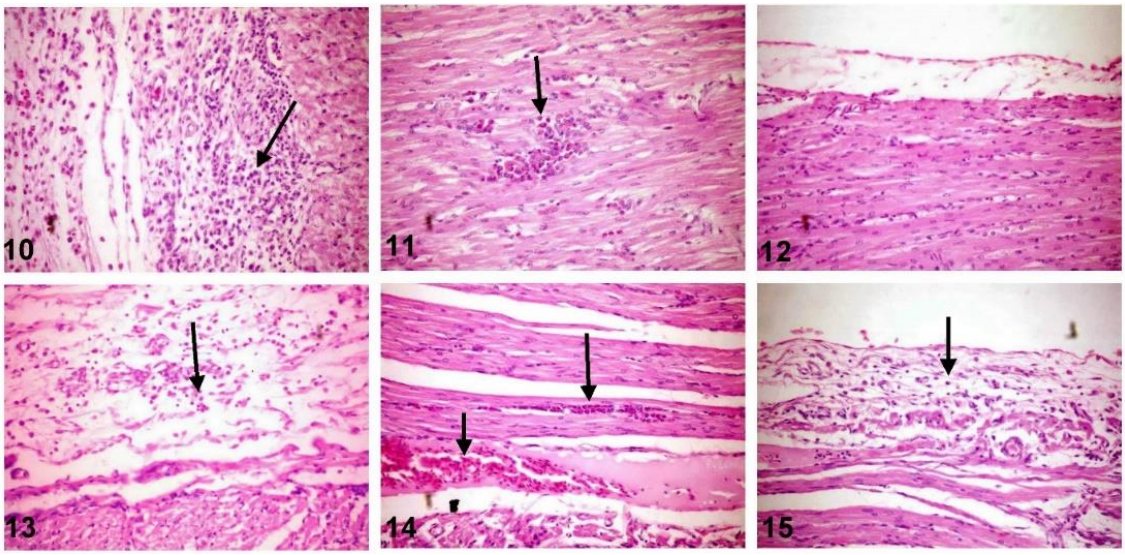

Plate 2: Heart of chickens infected with *E. coli* (Figs10-12) or *Salmonella Enteritidis* (Figs. 13-15). Fig. (10): showing severe pericarditis described by thickening of the pericardial membrane with fibrinous exaudate, homogenous eosinophilic caseated material, mononuclear leucocytes and heterophils infiltration (H & E stain X 100). Fig. (11): showing focal heterophilic cells aggregation were observed in cardiac myocytes (H & E stain X 200). Fig. (12): treated with cephardine showing no changes (H & E stain X 200). Fig. (13): showing moderate pericarditis described by pericardial edema and leucocytic inflammatory cells infiltration (H & E stain X 200). Fig. (14): showing congestion of myocardial blood vessel (short arrow) and intermyocardial heterophils infiltration (long arrow). Fig. (15): treated with cephardine showing mild pericarditis (H & E stain X 200).

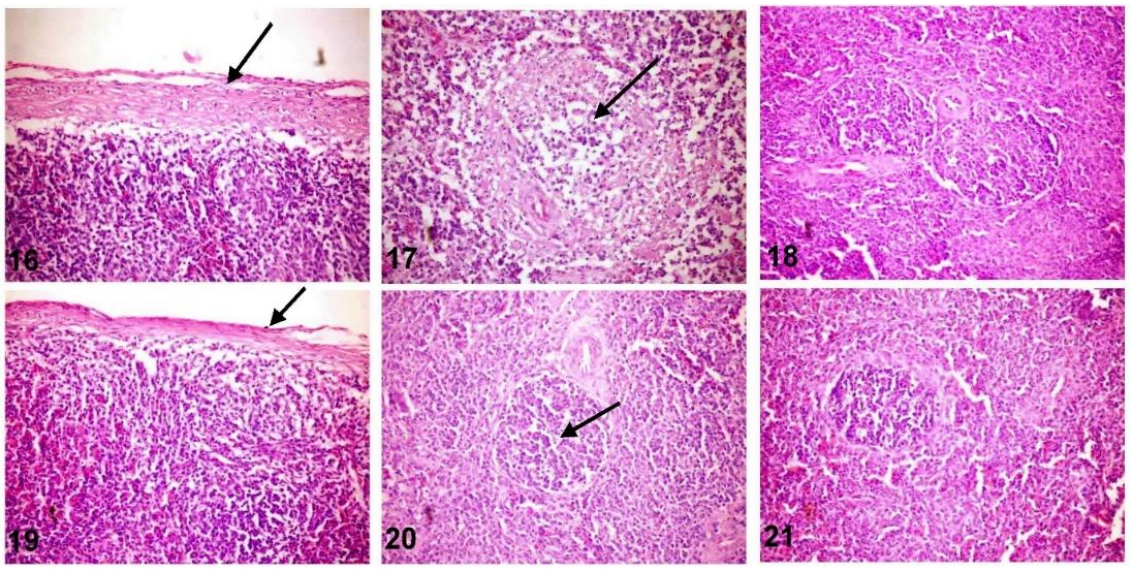

Plate 3: Spleen of chickens infected with *E. coli* (Figs 16-18) or *Salmonella Enteritidis* (Figs. 19-21), (H & E stain X 200). Fig. (16): showing Perisplenitis. Notice, thickening of splenic capsule with leucocytic cells infiltration (arrow). Fig. (17): showing marked lymphocytic necrosis and depletion (arrow). Fig. (18): treated with cephardine showing apparent normal lymphoid follicles. Fig. (19): showing slight thickening of splenic capsule (arrow). Fig. (20): showing slight lymphocytic necrosis and depletion (arrow). Fig. (21): treated with cephardine showing no histopathological alterations.

sections from chicken infected with salmonella and treated with cephardine revealed no histopathological changes (Fig. 8), whereas, other sections from this group showed portal infiltration with mononuclear leucocytes and heterophiles together with focal hepatic necrosis associated with massive heterophilic cells infiltration (Fig. 9).

Heart

Examination of sections from chicken infected with E. coli revealed severe pericarditis described by thickening of the pericardial membrane with fibrinous exaudate, homogenous eosinophilic caseated material, mononuclear leucocytes and heterophils infiltration (Fig. 10). In addition, subpericardial myocarditis and focal heterophilic cells aggregation were observed in cardiac myocytes (Fig. 11) of infected birds. Meanwhile, no histopathological changes were observed in the heart of with E. coli infected and treated group (Fig. 12). Concerning, heart of chicken infected with salmonella, it revealed from mild to moderate pericarditis described by pericardial oedema and leucocytic inflammatory cells infiltration (Fig. 13). Congestion of myocardial blood vessel and intermyocardial heterophils infiltration (Fig. 14) as well as perivascular oedema were also observed in some examined sections. However, heart from group infected with salmonella and treated with cephardine revealed improvement in the histopathological picture, as examined sections showed mild pericarditis (Fig. 15).

Spleen

Microscopically, spleen of chicken infected with *E. coli* showed perisplenitis described by thickening of splenic capsule with leucocytic cells infiltration (Fig. 16). Moreover, examined sections showed subcapsular oedema as well as lymphocytic depletion. The splenic parenchyma was infiltrated with massive heterophiles together with marked lymphocytic necrosis and depletion were noticed in all examined sections (Fig. 17). On the other hand, spleen of chicken infected with *E. coli* and treated with cephardine revealed no histopathological changes with apparent normal lymphoid follicles (Fig. 18). Examined sections of chicken infected with salmonella showed no alterations except slight thickening of splenic capsule (Fig. 19) with slight lymphocytic necrosis and depletion (Fig. 20). Moreover, no histopathological alterations were noticed in sections from chicken infected with salmonella and treated with cephardine (Fig. 21).

Several earlier studies indicated similar histological lesions for salmonellae (Barrow *et al.*, 1987, 1996 Mamun *et al.*, 2011; Gast, 2013) and for *E. coli* infection by (Cheville and Arp 1978, (Nolan *et al*., 2013).

Conclusions

Taking in consideration the aforementioned results, it could be concluded that cepharidine reduced the disease picture of colibaccillosis and salmonellosis. Eventually it could be successfully used in controlling such potent pathogens in chickens.

Authors contribution

GA AbdelAlim: Research Plan, experimental design, *E. coli and Salmonella* challenge, monitoring clinical signs and writing of the manuscript. Kawkab A. Ahmed: Histopathology assay and postmortem lesion scores.

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