International Scholars Journals

Advanced Journal of Microbiology Research ISSN 2241-9837 Vol. 13 (3), pp. 001-006, March, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Renal tissue damage due to *Eimeria coecicola* infection in rabbits

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Accepted 19 January, 2019

Coccidiosis causes considerable economic loss in the poultry industry. The study was designed to investigate the induced damage in renal tissue of rabbits infected with *Eimeria coecicola* sporulated oocysts. Animals were divided into two groups. The first group acted as the non-infected control group while the second group was infected with 50,000 *E. coecicola* sporulated oocysts. Infection which induced a weight loss and rabbits output were approximately 1.2 billion oocysts/g faeces on day 7 postinfection. Histological examinations revealed that the renal tissues of the infected animals were damaged, where the urinary space appeared wider, and some kidney tubule cells were vacuolated and the nuclei appeared to be slightly swollen than normal. Both carbohydrates and protein content in the infected renal tissue were reduced. Also, the level of both of urea and glucose in blood plasma were elevated due to infection with *E. coecicola* sporulated oocysts and reached 25.7 ± 1.1 and 143.8 ± 7.1 mg/dl, respectively. The results obtained from this study suggest that *E. coecicola* infection induced renal tissue damage.

Key words: Coccidiosis, renal tissue, rabbit.

INTRODUCTION

Coccidiosis, caused by species of intracellular protozoan parasites belonging to the genus *Eimeria* (Phylum: Apicomplexa), remains one of the economically most important diseases in modern poultry production (Dkhil et al., 2011). Rabbit coccidiosis is considered to be the most threatening factor affecting rabbit production (Peeters et al., 1984; Jithendran and Bhat, 1996) as it causes severe pathological changes to infected animals leading finally to huge economic losses in industrial rabbit farms (Baker, 2007; Taylor et al., 2007). Among the eleven Eimerian species infecting rabbits, *Eimeria coecicola* which infect mucosal associated lymphoid tissues (Bhat et al., 1996). Such parasite has an unusual mode of life cycle than other eimerian species infecting rabbit, as sporozoites take an extra intestinal way to reach their final site of multiplication (Pakandl et al., 2006), and such sporozoites were found in other organs such as spleen, mesenteric lymph nodes and peripheral blood vessels (Renaux et al., 2001). The appendix tissue has been described to undergo structural changes in response to *E. coecicola* infection (Vitovec and Pakandl, 1989). Presumably involving changes due to the inflammatory response of rabbits to *E. coecicola*. Intestinal eimerian infection can alter and affect other organs than their final sites of multiplication. For example degenerative changes occurred within renal tissue of turkey infected with *E. adenoides* (Lozanov and Koinarski, 1985) and it was attributed to the toxic cell debris in the intestine. Also, in lambs suffering from intestinal coccidiosis a membrane proliferative glomerulonephritis was reported due to trapped antigen/antibody complexes by kidney glomeruli (Majid and Winter, 1986).

Many previous studies provoked that there is a disturbance in ion absorption and excretion as Na^+, Cl^- and K^+ during intestinal coccidial infection (Fitzgerald, 1967; Mazurkiewicz et al., 1986; Turk, 1986). These disturbances in ion concentration affect the renal tissue.

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In *E. coecicola* infection, the kidney of rabbits has not yet been investigated to date. This prompted us to characterize the pathological effects of such parasite on kidney of rabbits at both tissue and blood scales.

MATERIALS AND METHODS

Animals

Twenty healthy female rabbits (*Oryctolagus cuniculus*) approximately 8 to 9 weeks old ranging in weight from 2.0 to 2.5 kg, obtained from the animal house of King Saud University were used in the present study, their faeces were examined daily for one week to assure the absence of any Eimerian infection. Animals were housed in special cages and were kept in the laboratory under constant conditions for at least one week before use. The experiments were approved by the state authorities and followed Saudi Arabian rules for animal protection.

Infection with *E. coecicola*

Animals were divided into 2 groups (10 animals each). The first group acted as the control non-infected group while the second group was the infected group with *E. coecicola* sporulated oocysts. *E. coecicola* used in this study is a pathogenic species of the small intestine. Oocysts were collected from faeces of rabbits naturally infected with *E. coecicola* and then surface sterilized with sodium hypochlorite and washed at least four times in a sterile saline solution prior to oral inoculation as described by Schito et al. (1996). These oocysts were used to inoculate rabbits by oral gavaging each rabbit with 50,000 sporulated oocysts of *E. coecicola* suspended in 1 ml sterile saline. Once every 24 h, fresh faecal pellets were collected and weighed for each rabbit and the bedding was changed to eliminate reinfection. Oocyst output was measured as previously described (Schito et al., 1996). Faecal pellets were suspended in 2.5% (wt/vol) potassium dichromate and diluted in saturated sodium chloride for oocyst flotation. Oocysts were counted in a McMaster chamber and expressed as number of oocysts per gram of wet faeces.

Histological and histochemical study

Animals from control and infected groups were sacrificed after 7 days of infection with *E. coecicola* sporulated oocysts. Small pieces of the kidneys were quickly removed, then fixed in neutral buffered formalin. Following fixation, specimens were dehydrated, embedded in wax, and then sectioned to 5µ thickness. For histological examinations, sections were stained with haematoxylin and eosin (Drury and Wallington, 1980). Histological damages were scored as follows: 0: absent; +: mild; ++: moderate; and +++: severe. For the histochemical study, sections were stained with periodic acid-Schiff's method to demonstrate total carbohydrates (Hotchkiss, 1948), and with mercuric bromophenol blue method to demonstrate total proteins (Maize et al., 1953).

Biochemical studies

Both of non-infected and infected rabbits were sacrificed on day 7 p.i. with *E. coecicola* sporulated oocysts. Blood was collected from the heart into heparinized tubes. Blood plasma was separated according to Dkhil (2009). Biochemical parameters in the blood plasma were analyzed using commercial kits (Biomerieux, Marcy l'Etoil, France) including: albumin, globulin (Gornall et al., 1949), urea (Fawcett and Scott, 1960) and glucose (Trinder, 1969) according to manual of manufacturer.

Statistical analysis

Statistical analyses were performed using Student's *t*-test at $p < 0.01$.

RESULTS

Rabbits infected with *E. coecicola* began with shedding of oocysts on day 5 p.i. (Figure 1). Maximal shedding of approximately 1.2 million oocysts/g faeces was on day 7 p.i. Symptoms of coccidiosis clearly appeared on day 7 p.i. where rabbits became weak and excrete watery mucoid diarrhea. Infection with *E. coecicola* induced a significant ($p \leq 0.01$) weight loss of about 800 g after 7 days from the infection (Figure 2). The normal histological structure of the kidney is shown in Figure 3A. The urinary space appeared wider in the infected group when compared to the control group (Figure 3B). Some blood sinusoids appeared to be filled with erythrocytes. Some kidney tubule cells were vacuolated and the nuclei appeared to be slightly swollen than normal. In general, infection with *E. coecicola* induced tissue damage as scored in Table 1. Tissue revealed a reduced amount of cytoplasmic glycogen, with diminished density of basement membranes, and brush borders of the kidney tubules (Figure 4B). Also, the glomeruli were less positive than those of the control group (Figure 4A). Proteins of the control kidney tubules and glomeruli showed homogeneously dense blue-colored granules due to a positive affinity to bromophenol blue staining (Figure 5A). In infected group, the protein content was slightly decreased in the kidney tubules and glomeruli (Figure 5B). The level of both albumin and globulin were non-significantly changed after the infection with *E. coecicola* (Table 2).

Both urea and glucose levels in blood plasma were significantly elevated due to infection with *E. coecicola* sporulated-oocysts and reached 25.7 ± 1.1 and 143.8 ± 7.1 mg/dl, respectively (Table 2).

DISCUSSION

The recorded renal tissue damage due to infection with *E. coecicola* in the present investigation may be discussed from the physiological point of view. The kidney is the main excretory organ of the body, so the elevation of the parasite residues concentration in the blood must be faced by capillary constriction to decrease the glomerular filtrate containing the parasite to minimize its effect and protect the tubular cells. At the same time,

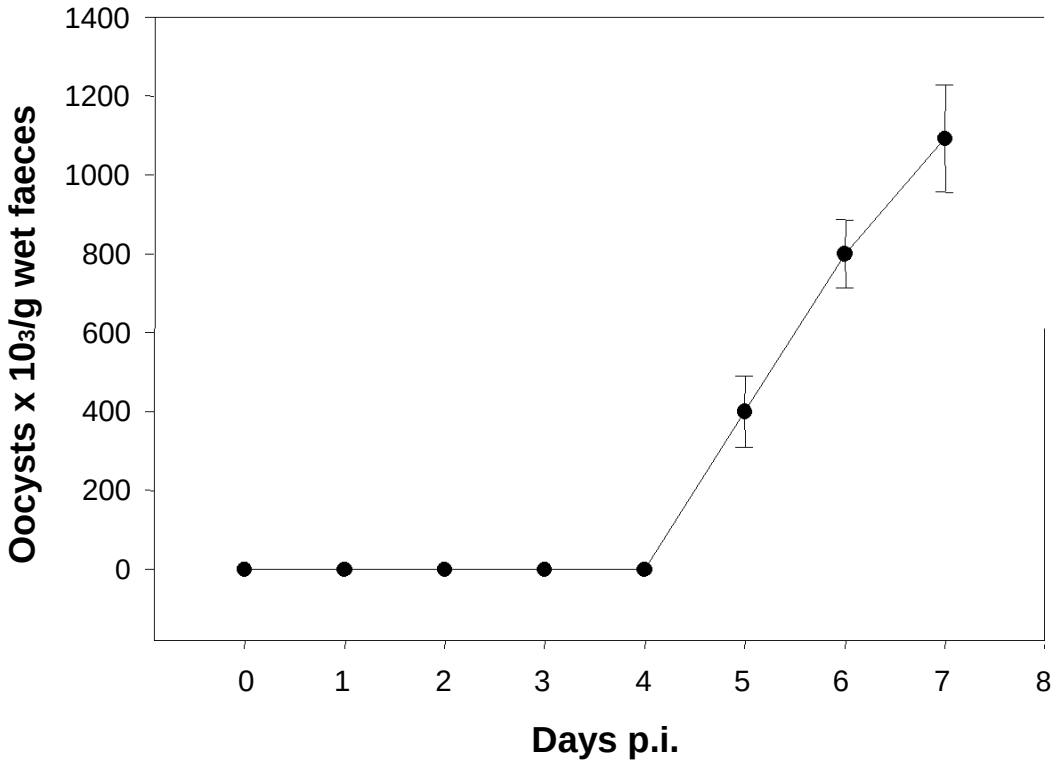

Figure 1. Oocysts output of rabbits infected with 50,000 sporulated *E. coecicola* oocysts. All values are means±SD.

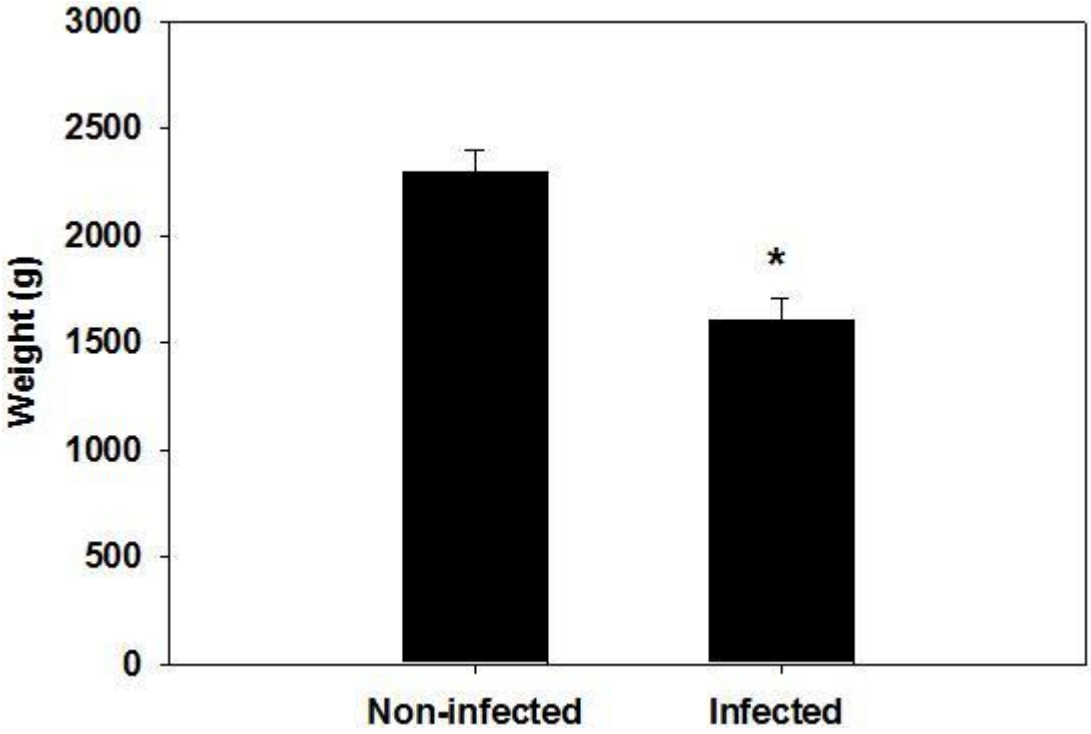

Figure 2. Change in weight of rabbits on day 7 postinfection with *E. coecicola* sporulated oocysts. All values are means±SD. *, significant change at p≤0.01.

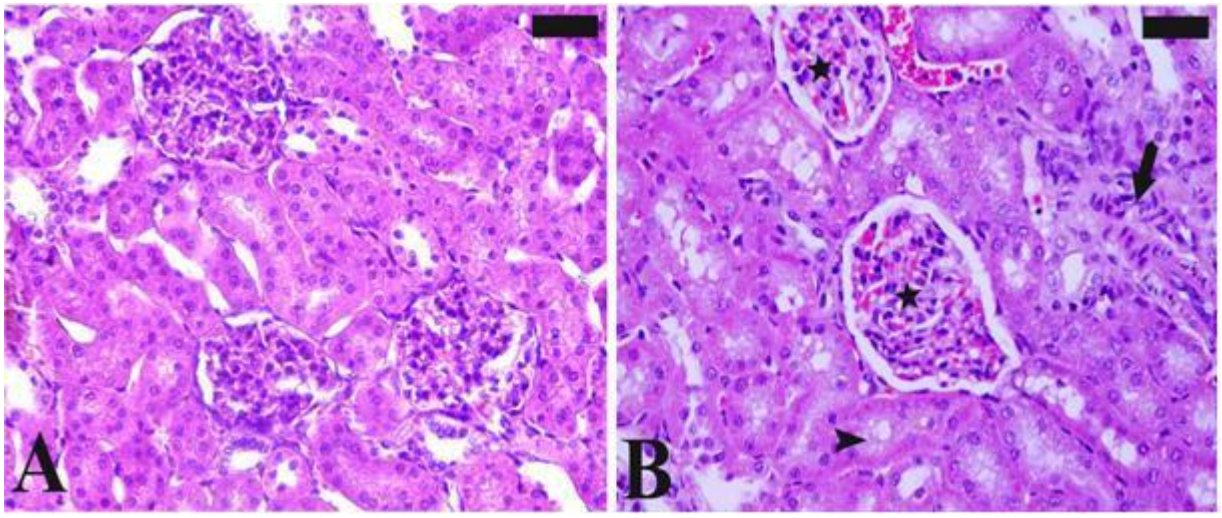

Figure 3. Histological changes in kidney of rabbit. A) Control kidney with normal architecture. B) Kidney sections of rabbit infected with *E. coecicola*-sporulated oocysts. Some kidney tubules are vacuolated (arrow head), Inflammatory cellular infiltrations appeared in some areas (arrow). Glomerulei contain more erythrocytes (star). Sections were stained with hematoxylin and eosin. Scale bar = 50 µm

Table 1. Histopathological kidney damages induced by *E. coecicola*.

Group	Tubular vacuolization	Microscopic observation		
		Hydropic degeneration change	Glomerular damage	Inflammatory cellular infiltration
Non-infected rabbits	+	0	0	0
Infected rabbits	++	+++	++	+++

0: absent; +: mild; ++: moderate; +++: severe.

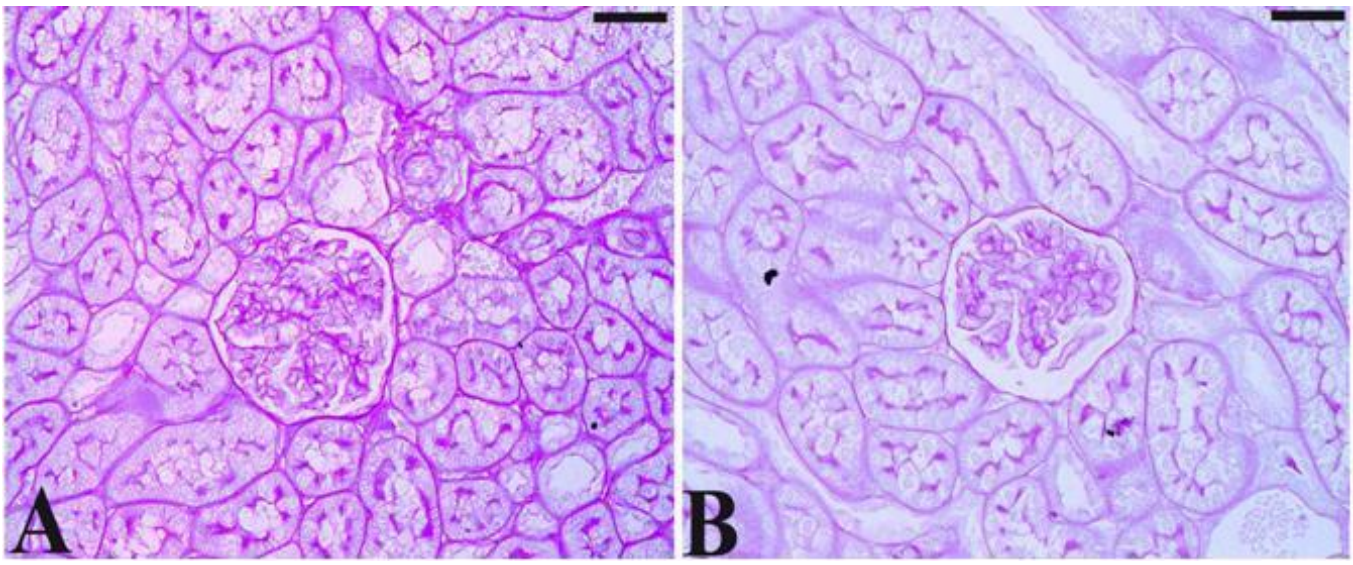

Figure 4. Total carbohydrates in kidney sections. A) Control kidney. B) Kidney sections of rabbit infected with *E. coecicola* -sporulated oocysts. Kidney appeared with a slight decrease in carbohydrate content. Sections were stained with periodic Schiff's method. Scale bar = 50 µm.

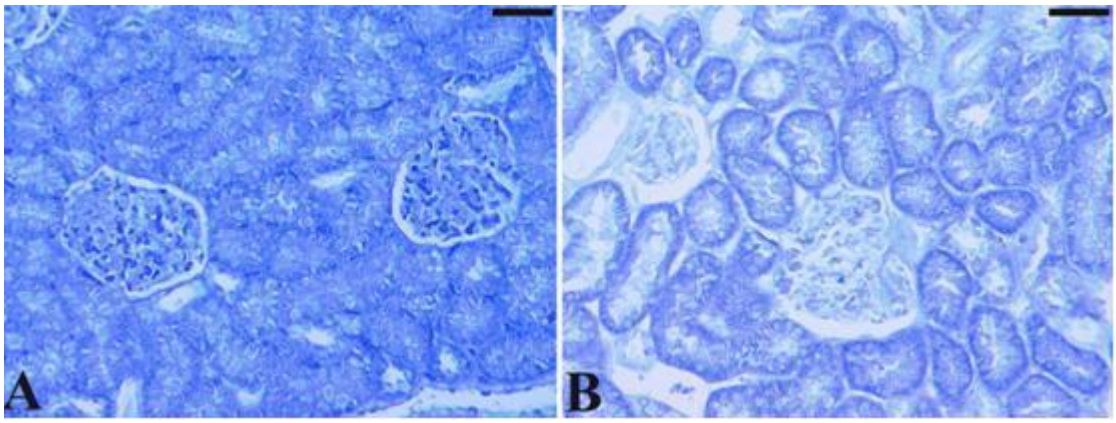

Figure 5. Protein content in kidney sections. A) Control kidney. B) Kidney sections of rabbit infected with *E. coecicola*-sporulated oocysts. Treated kidney section with a weak response towards bromophenol blue reaction. Sections were stained with bromophenol blue method. Scale bar = 50 µm.

Table 2. Changes in albumin, globulin, urea and glucose in plasma of rabbits due to infection with *E. coecicola*.

Group	Albumin (g/dl)	Globulin (g/dl)	Urea (mg/dl)	Glucose (mg/dl)
Non-infected	4.6 ± 0.4	2.7 ± 0.2	14.4 ± 1.1	121.1±8.1
Infected	4.3 ± 0.3	2.4 ± 0.4	25.7 ± 1.1*	143.8±7.1*

Values are means ± SD. *, Significant change at $p \leq 0.01$.

the mesangial cell processes may be retracted due to the contraction of their filaments (myosin-like filaments) which may be stimulated by angiotensin II present in these cells (Stevens and Lowe, 1997). The altered mesangial cellularity may increase its phagocytic function to clear some of the parasite residues from the circulating blood, and also secrete more angiotensin II to constrict the glomerular capillaries, slowing the blood flow to decrease the glomerular filtrate, so a minimum amount of parasites residues reaches the tubular lumen with the glomerular filtrate and in the blood capillaries surrounding these tubules (Wunschmann et al., 2010). The tubular lesions observed in the present study were accompanied by invasion of inflammatory cells to the intertubular tissues in a trial to minimize the injury. Some of these external stressors apparently caused the tubular lesions. Renal tubules appeared with cytoplasmic vacuolation which is mainly a consequence of considerable distur-bances in lipid inclusions and fat metabolism occurring under pathological cases (Zhang and Wang, 1984; Ebaid et al., 2007). The decrease in carbohydrate content was attributed by some investigators to be due to increased stress on organs, leading to high energy consumption which allowed an equalized pressure to be exerted upon them (Ebaid et al., 2007). The decrease in protein could be attributed to the disruption of lysosomal membranes under the effect of various toxicants leading to the liberation of their hydrolytic enzymes in the cytoplasm resulting in marked lysis and dissolution of the target material (Ebaid et al., 2007).

The elevated serum glucose level observed may have resulted from increased mobilization of glucose for metabolism or may be due to reduced glucose uptake into cells (Bray et al., 1999) caused by infection. This is also suggestive of a possible modulation of the capacity of the renal tubule by the infection to reabsorb glucose actively from the blood (Bray et al., 1999). *E. coecicola* infection induced higher serum urea concentration may impair the secretory function of the kidney (Whealton et al., 1994). Malfunction in the glomerular filtration results in the retention of substances including urea, and this may be responsible for their high serum levels in the infected group. The results obtained from this study suggest that *E. coecicola* infection induced not only intestinal damage but also renal tissue damage. This affects the rabbit pathology and affects the poultry production.

ACKNOWLEDGMENT

The author extends his appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project No. RGP-VPP-002.

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