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Epidemiological, Morphological, and Histopathological Study of Quail Coccidiosis in Thi-Qar Province, Iraq

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ABSTRACT

Coccidiosis is the most common and important disease of poultry resulting in great economic losses world widely. The objective of this study was to define the species of *Eimeria*, their prevalence, and gut histopathological lesions in quails (*Coturnix coturnix japonica*) in Thi-Qar province, Iraq. Over the period of ten months (December 2019 to September 2020), a total of 330 fresh fecal samples (201 from males and 129 from females) were collected from different marketplaces and farms in Thi-Qar province. Direct smear and flotation techniques were used to examine the presence of *Eimeria* oocysts in the fecal samples. The sporulated oocyst shape indices were measured microscopically. Hematoxylin and eosin, as well as Periodic-Acid Schiff stains were used to diagnose potential pathological lesions and *Eimeria* developmental phases within the small (duodenum, jejunum, and ileum) and large (caeca) intestines. Four species, according to the criteria adopted, *Eimeria bateri*, *Eimeria uzura*, *Eimeria tsunodai*, and *Eimeria fluminensis* were uncovered in current study, with shape indices of 1.30, 1.36, 1.30, and 1.05, respectively. The total infection rate was 64.54%; and the morphological characteristics of the oocysts was identified. The highest infection rate was in *E. bateri* (41.78%), while the lowest infection rate was in *E. fluminensis* (7.98%). The highest prevalence rate was in March 84.84% with a significant effect at $P<0.01$. The sex of quail had no significant effect of the coccidiosis infection. The study revealed a destruction in the epithelium of the mucosa and submucosa of the intestine and development of the *Eimeria* stages, which led to malabsorption and consequently weight loss and economic losses in the quail industry.

Keywords: coccidiosis, quail, morphological, histopathological, epidemiological

INTRODUCTION

Quails are most susceptible for various diseases such as coccidiosis which is recognized as a dangerous parasitic disease problem limiting the quail industry (1). Avian coccidiosis is an enteric parasitic disease is caused by multiple species of the protozoan parasite of genus *Eimeria*. *Eimeria* is a single-cell, obligatory intestinal epithelial parasite that is one of the most common and cost-effective poultry diseases in the world, causing substantial morbidity and mortality rates (owing to acute and bloody enteritis) (2-4). In Iraq, (5) recorded species of *Eimeria* (*E. tsunodai*, *E. uzura*, and *E. bateri*) in AL-Mosul, while in Baghdad (6) recorded four species of *Eimeria* (*E. bateri*, *E. tsunodai*, *E. uzura*, and *E. fluminensis*) in quail birds. The mechanism and degree of tissue damage depended on the species of *Eimeria* involved, the size of the infectious dose of oocytes, stress, and various factors related to the host including age, physical condition, genetic sensitivity, and degree of immunity in young animals (7, 8). The pathological changes

of intestines and caecum differed according to the various species for *Eimeria*. In most cases, loss of surface epithelial cells and atrophied villous associated with first generation schizonts, while crypt destruction or hyperplasia was associated with gametogons stages. Histopathological lesions in the case of the caecal form revealed loss of epithelial tissue, congestion of blood vessels. Severe muscular oedema, loss of villi. Disruption of caecal mucosa, a cluster of oocysts and marked haemorrhage, necrosis of caecal mucosa and lymphoid cells showing hyperplasia (9, 10). This study was conducted to find out the prevalence *Eimeria* spp and potential pathological changes in quails.

MATERIALS AND METHODS

Sample Collection

All procedures carried out in this study were reviewed and accepted in compliance with the ethical principles of animal welfare by the Scientific Committee at the College of Veterinary Medicine, University of Bagdad.

Fecal samples were collected from 330 Quails birds (*Coturnix coturnix japanica*) from birds selling markets and farms of both sexes (201 males and 129 females from different regions in Thi-Qar province. It started from December 2019 to September 2020. The samples were transferred in a cool box to the laboratory of parasitological department, at the College of Veterinary Medicine, University of Baghdad for microscopical examinations.

Laboratory Techniques

Fecal samples were examined for the presence of coccidiosis oocysts using the direct smear and flotation techniques as described by Charles and Robinson (11). Oocyst positive samples were diluted into 2.5% potassium dichromate (BHD/UK) and kept in Petri dishes for sporulation at room temperature (12).

The shape indices (length/width) of the sporulated oocysts were determined using the method for species identification (13). Calibration was done using Ocular Micrometer-the calibration of a microscope for oocyst measuring (14).

Histopathological Examination

The birds were euthanized under guides for the care and use of laboratory animals. Section of the parts of the small intestine including the caecum were taken for histopathological studies (15).

Tissue sections were taken from the different regions of intestinal section (Duodenum, jejunum, ileum, and caecum) of natural infected birds. Sections of tissue put in formalin of 10%, Then transferred to the histocaint device to pass in ethanol alcohol (70%, 80%, 90% and absolute). Then it is immersed in xylol solution for two phases then a process of casting molds and wax models were done then obliterated in paraffin. The samples were cut into strips of tissue with a thickness of 5 µm by microtome device. Tissue slides were stained by hematoxylin & eosin and PAS stain (15, 16).

Statistical Analysis

The Statistical Analysis System program (SAS) was used to determine the effect of different factors. Chi-square (χ^2) test was used to compare between groups in the study at 0.05 and 0.01 probability (17).

RESULTS AND DISCUSSION

The study revealed that 213 cases out of a total of 330 fecal samples were infected with *Eimeria* species, with a total infection rate of 64.54%. Our result agreed with (18) who recorded an infection rate of 62.5% in Egypt. Similarly, the result in Ethiopia was 65.10% (19). In Iraq, previous studies were recorded different infection rates of *Eimeria* in Quails. For instance, Mohammad (5) recorded a 49.4% infection rate in Mosul city which was lower than the results in the present study while (6) recorded a 78.33% infection rate in Baghdad, which was higher than the results of our study. The difference in the *Eimeria* infection rates could be attributed to the number of samples, the density of the birds, type of rearing of the birds such as (overcrowding) and environmental condition (temperature and humidity) may lead to increase the infection rate.

This study demonstrated the morphological characteristics and structure of sporulated oocysts (Table 1) by microscope to determine the species of *Eimeria* based on previous studies (6, 20-24). Four species were recorded in this study of *Eimeria* in quail were *E. bateri* (Figure 1), *E. uzura* (Figure 2), *E. tsunodai* (Figure 3), and *E. fluminensis* (Figure 4).

Our results were incompatible with many researchers (6, 20-23). The diagnosis of coccidiosis, species description and systematics, were all dependent upon the characterization features of the oocyst; morphological characteristics emphasized that the applications of molecular methods used for sporulated coccidian oocysts (24).

Infection Rate of *Eimeria* Species in Quail

This study showed that *Eimeria bateri* had the highest infection rate (41.78%) compared to other species, while *Eimeria fluminensis* had the lowest infection rate (7.98%), with a significant difference of $P<0.01$ (Table 2). This result was in agreement with (5) who found that *E. tsunodai* had an infection rate of 44.8%, *Eimeria bateri* had a 24.1%. and *E. uzura* 34.5%. While agreed with (6) who recorded that the highest infected rate was with *E. bateri* (66.11%) but disagreed in the lower infected rate where recorded *Eimeria uzura* to be 23.88%. These investigations could be

attributed to the different areas that samples were collected, quail rearing and reproduction system, the numbers of the samples and environmental conditions

Infection Rates of *Eimeria* According to Months

The results during the months of the study showed a significant difference at P≤0.01. March and April recorded higher infection rate which was 84.84% and 78.78 %, respectively. While the lower infection rate was 27.27% in July (Table 3). These results were incompatible with previous studies reported that an increase in infection rate during the spring months and a decrease in summer months in chickens in Al-Muthanna province, Iraq. (25, 26) similarly found that the study on the prevalence of coccidian in Chickens, Quails and Wild Pigeon in Pakistan recorded high prevalence rates of coccidian in spring months (27). Our study was incompatible with previous observations of the authors (28) who showed a higher infection rate in July (94.4%) followed by June (57.9%), respectively (29) who showed a higher infection rate in January (91.4%) but a lower infection rate in April (44.1%) indicated to the high rates of infection in January and February to be 90% and 86.66%, respectively (6).

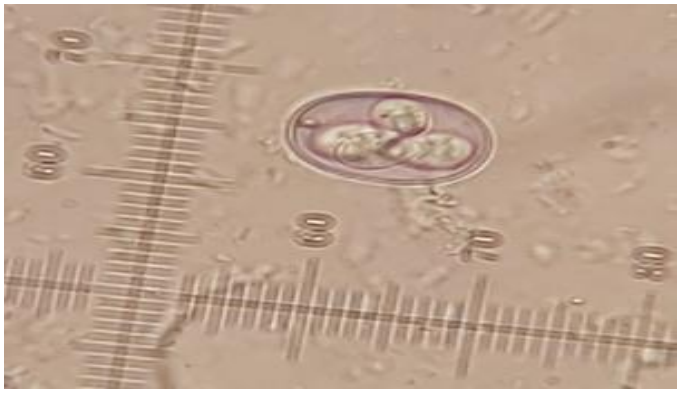

Figure 1. Sporulated oocyst of *Eimeria bateri* (direct wet smear, 40×)

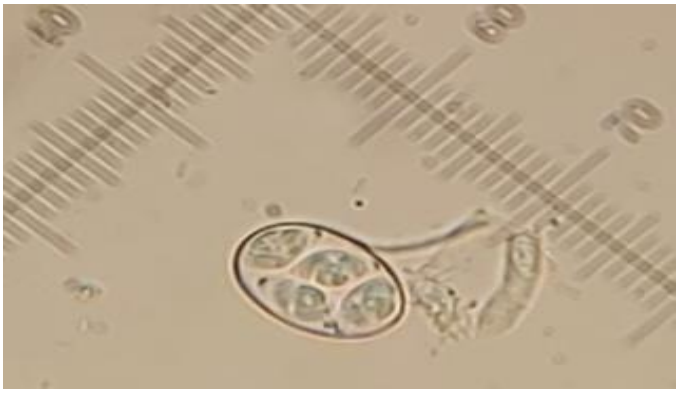

Figure 2. Sporulated oocyst of *Eimeria uzura* (direct wet smear, 40×)

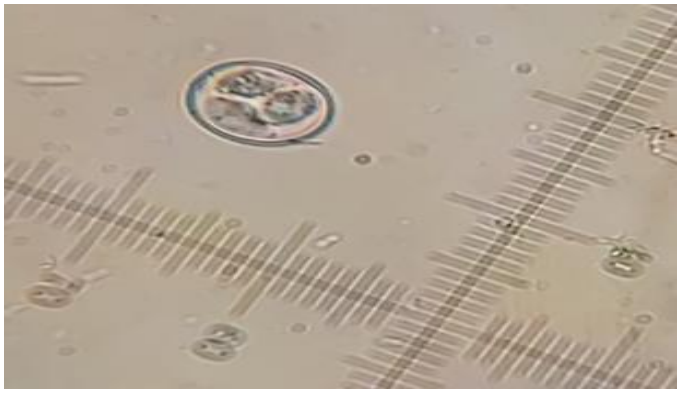

Figure 3. Sporulated oocyst of *Eimeria tsunodai* (direct wet smear, 40×)

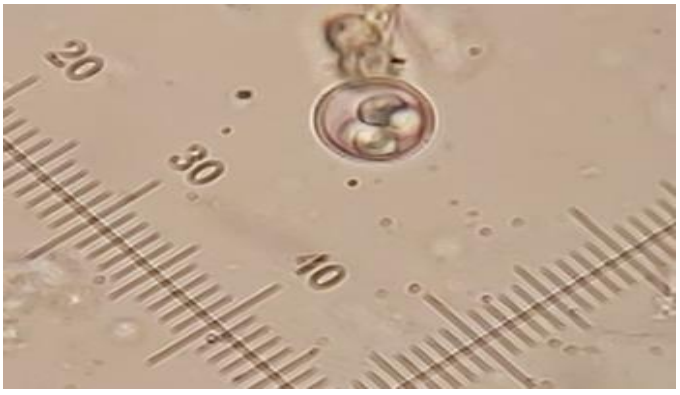

Figure 4. Sporulated oocyst of *Eimeria fluminensis* (direct wet smear, 40×)

Table 1. Characteristic features and measurements of sporulation oocyst of *Eimeria* spp. in quail

Feature	*Eimeria* spp			
	E. bateri	***E. uzura***	***E. tsunodai***	***E. fluminensis***
Shape	Sub spherical to ellipsoidal	Ovoid to ellipsoidal	Ovoid to sub spherical	Spherical to sub spherical
Range (L × W)	18-31 × 14-26	19-26 × 15-19	18-24 × 14-19	15-18.75 × 15-17.5
Shape Index	1.30	1.36	1.30	1.05
Polar granule	+	+	+	-
Micropyle	-	-	-	-
Shape sporocyst	Ovoidal	Ovoidal (elongated)	Ovoid to ellipsoidal	Ovoidal
Shape Stied a body	Nipple-like	Half-moon or knob-like	Nipple like to triangular	Present

Table 2. Infection rate of *Eimeria* spp. in quail

Eimeria spp	Total No.	No. *Eimeria* spp	% Eimeria spp
E. bateri	213	89	41.78*
E. uzura		61	28.63
E. tsunodai		46	21.59
E. fluminensis		17	7.98*
χ^2			12.674

*P≤0.01

The prevalence relationship of infection rate was positively influenced by the warm and humid weather, which were characterized by rainy season as favorable conditions for the growth and development of infective oocysts. The poor management of poultry can exacerbate the problem and result in great economic losses. Besides, the spring season is considered the reproduction season for the most animals which increase the activity and raise the chance of exposure to infection (25, 27).

Table 3. Infection rates of *Eimeria* spp. in quail according to months of the study

Months	No. Samples examined	No. Positive	% Positive
December	33	21	63.63
January	33	24	72.72
February	33	25	75.75
March	33	28	84.84*
April	33	26	78.78
May	33	21	63.63
June	33	23	69.69
July	33	9	27.27*
August	33	15	45.45
September	33	21	63.63
Total	330	213	64.54
χ^2			13.214

*P≤0.01

Infection Rates of *Eimeria* According to Quail Sex

This study showed a high infection rate of (67.16%) in males, whereas the females represented a low rate of infection (60.46%) with insignificant at (P>0.05) (Table 4).

Table 4. Infection rates of *Eimeria* according to quail sex

Sex	No. Sample examined	No. Positive	% Positive
Males	201	135	67.16*
Females	129	78	60.46*
Total	330	213	64.54
χ^2			2.091

*P≥0.05

The prevalence of *Eimeria* spp was not affected by the sex of quail showing a high rate of infection in males than females possibly the majority of the males were adult. These results were in agreement with Liburd (30) in California who found non significant difference (P≥0.05) with a higher infection in males. In Iraq, previous studies recorded a higher infection rate in males (50%) than females (48.9%) without significant difference at (P>0.05%) of coccidian infection (15, 16). In contrast, another study in Iraq recorded the highest rate in males (83.01%) than females (71.61%) with significant differences at (P<0.01) (5). It can be said that these differences in the relationship of the host's sex to the parasite are relative since they vary depending on the type of host, sometimes the infection rates in males are higher than that of females, and sometimes it occurs conversely. It is not possible to confirm the existence of sex-related determinants that affect parasite infection and make it tilt to a specific sex of a host without the other. As we do not rule out that, some cases led to immune suppression for females during egg-laying and incubation period of female birds (27, 30).

Histopathological Changes

The tissue sections from small intestine and caecum revealed significant pathological changes, thickening the epithelial cells and hypertrophy of the villi with crypt enlargement, resulted from heavy parasitic infection reflected the presence of different stages of *Eimeria* spp mostly occur in parts of the small intestine as duodenum (Figure 5), jejunum (Figures 6 and 7), and ileum (Figure 8). The histopathological changes of the caecum were characterized by parasitic vacuoles in the mucosal epithelium and lamina propria with aggregation of micro and macrogametes in the submucosa and desquamation of surface epithelium (Figures 9 and 10).

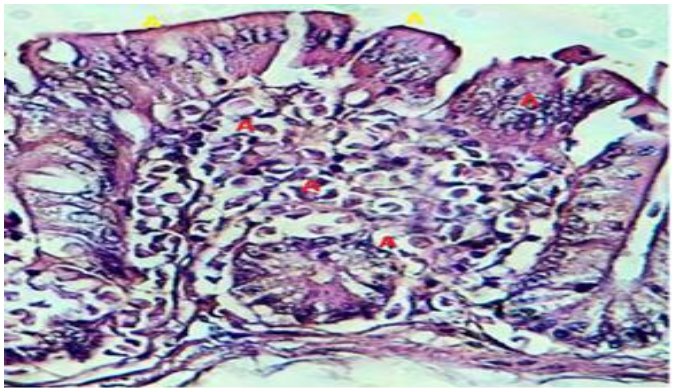

Figure 5. Histopathologic section of duodenum shows, thickening the lining epithelial cells of the villi (A), heavy infection with *Eimeria* merozoites in the mucosa and lamina propria (A), (H&E stain, 40×)

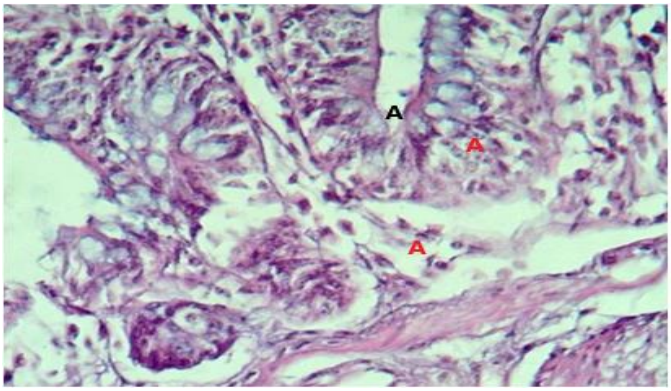

Figure 6. Histopathologic section of jejunum shows, different stages of *Eimeria* spp in enterocytes (A), crypt hyperplasia (A), (H&E stain, 40×)

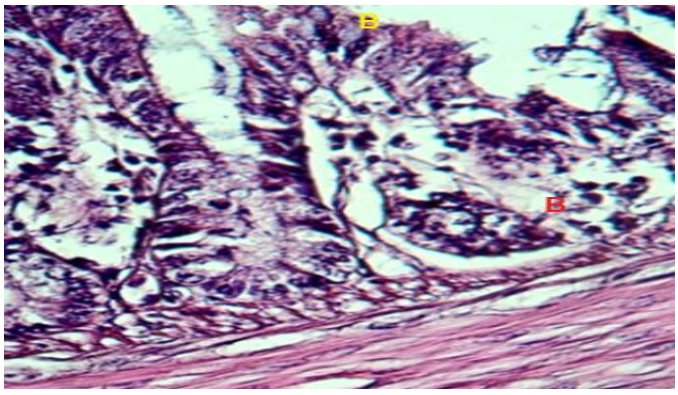

Figure 7. Histopathologic section of jejunum shows, villous hypertrophy (B), different stages of *Eimeria* spp (B), (H&E stain, 40×)

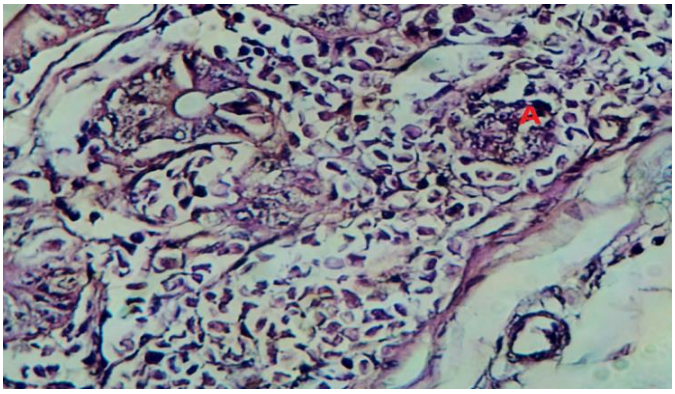

Figure 8. Histopathologic section of ileum shows, schizonts rupture and release merozoites in lamina propria (A), (PAS stain, 40×)

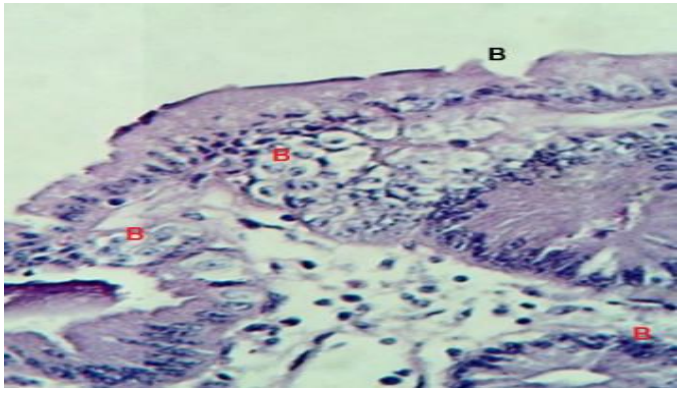

Figure 9. Histopathologic section of caecum shows, desquamation of surface epithelium (**B**), parasitic vacuoles in surface epithelium and lamina properia aggregation of (micro and macrogametes) (B), (H&E stain, 40×)

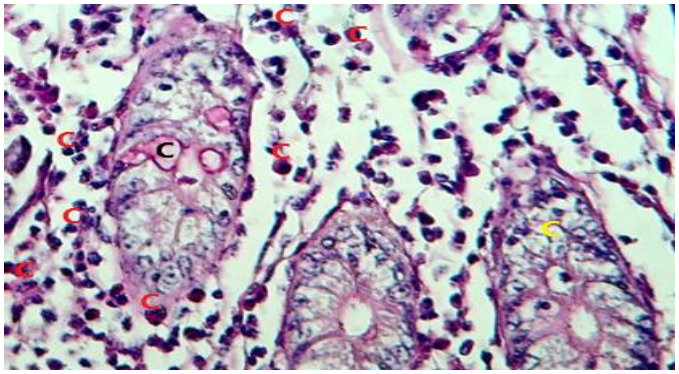

Figure 10. Histopathologic section of caecum shows red color for mucin from goblet cells (**C**), Infiltration of inflammatory cells eosinophils and heterophils (**C**), Crypt cells enlargement due to presence of different stages of *Eimeria* spp (C), (PAS stain, 40×)

These histopathologic lesions were characterized by severe necrotic enteritis described as enlargement intestinal villi and their fusion resulted from massive erosive of the small intestine. Heavy infiltration of inflammatory cells occasionally eosinophils in lamina propria extended to submucosa in caecum sometimes to muscular coat and reach to serosa.

The intestinal villi showed severe degenerative of enterocytes and necrotic changes range from hydropic, swollen cells sometimes filled with parasitophorous vacuoles of protozoal developmental stages. Parasitophorous either uninucleated the epithelial cells, besides released free merozoites from enterocytes mostly in crypts.

Increased production of mucinous material as red color filled the goblet cells between villous epithelial cells and in the crypt-mucosal epithelium.

A common feature of the infection was a severe depression of the digestive and absorptive capabilities of the mucosa. Furthermore, the magnitude of all disturbances and functional changes were usually related to the intensity of the parasitic infection (31, 32). The study revealed a distraction in the epithelium of the mucosa and submucosa of the intestine and development of the *Eimeria* stages in it, which led to malabsorption and consequently to weight loss and economic losses in the quail industry.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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دراسة وبائية ومورفولوجية ومرضية لمرض الكوكسيديا في طيور السمان في محافظة ذي قار، العراق

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الخلاصة

تعد الكوكسيديا من الأمراض الشائعة في الدواجن والتي تسبب خسائر اقتصادية كبيرة . اجريت هذه الدراسة من كانون الاول 2019 الى ايلول 2020. جمعت 330 عينة براز من طيور السمان من اسواق بيع الطيور ومزارع السمان في محافظة ذي قار، العراق. بلغ اجمالي نسبة الاصابة 64,54%. تم التعرف على الصفات الشكلية لكيس البيضة، كان اعلى معدل اصابة 41,78% في (*E. bateri*) بينما اقل معدل اصابة كان 7,98% في (*E. fluminensis*). كان اعلى معدل انتشار في اذار 84,84% مع تأثير معنوي عند (P≤0.01). لم يكن لجنس السمان أي تأثير معنوي لعدوى الكوكسيديا. كشفت الدراسة عن وجود تحطم في ظهارة الغشاء المخاطي وتحت المخاطية للأمعاء وتطور مراحل الأميريا فيها مما يؤدي إلى سوء الامتصاص وبالتالي فقدان الوزن والخسائر الاقتصادية في صناعة السمان.

الكلمات المفتاحية: الكوكسيديا، السمان، الصفات الشكلية، النسج المرضي، الوبائية