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PREVALENCE OF COCCIDIOSIS IN CHICKEN IN SOHAGE GOVERNORATE

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Received: 2 September 2021; **Accepted:** 18 September 2021

ABSTRACT

Eimeria sp. is one of the most important parasites that cause very high economic loss in poultry farms in Egypt. It causes a disease called coccidiosis. This study aimed to detect the prevalence of *Eimeria* species in chicken and detection of its pathological effect within the intestinal mucosa. The present study investigate the prevalence of *Eimeria sp*. in chicken through fecal examination and the diagnosis was based on direct fecal sample examination (unstained wet mount technique) and concentration techniques**,** followed by sporulation of unsporulated oocyst for identification of *Eimeria spp*. and finally studying the pathological effect of this parasite in the intestinal mucosa of infected chicken. The total prevalence rate of *Eimeria spp*. was **(**66%). The incidence rate in Broiler chickens was (70%) and in Balady was (58%). The highest percent of infection was at the age of (15-30) day (54.3% in Broiler and 72.4% for Balady), and the disease was more prevalent in winter than in summer. The species that were detected are *E.acovullina* (the highest prevalence rate) followed by *E.tenella, E.necatrix* followed by *E.mitis* (lowest prevelance rate). These results indicate that the coccidiosis is a serious parasitic disease that effect on the poultry production in Egypt and control measures should be put in consideration to overcome this disease.

Keyswords: *Eimeria* - prevalence- coccidiosis- oocyst.

INTRODUCTION

Chickens represent the biggest poultry sector that reared intensively and represent a good, cheap and healthy protein source. Among all parasites of poultry, *Eimeria* are the most economically significant parasite (Blake *et al.,* 2020). *Eimeria* caused a disease that called coccidiosis, that is widely spread avian disease particularly in chickens either commercial or rural that can seriously affect the development of poultry production (Bachaya *et al*., 2015).

Coccidiosis is endemic in most of the tropical and subtropical regions where ecological and management conditions are suitable for sporulation of coccidian oocyst and development (Blake and Tomley, 2014). The genus *Eimeria* is a protozoan within the taxonomic family Eimeriidae, class Coccidia, order Eucoccidiorida and the phylum Apicomplexa. It is obligate intracellular parasites that transmitted to new host cells by invasive extracellular stages

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(sporozoites), which are equipped with a certain structure known as apical complex that aid in penetration of the host cell (Pastor-Fernandez *et al.,* 2018).

There are 9 *Eimeria species* are infective to chickens, identified as *E. acervulina, E. mivati, E. maxima, E. necatrix, E. brunitti, E. pracox, E. tenella,* and *E. hagani* (Hamid *et al.,* 2018). *E. tenella* is a highly pathogenic with heavy oocysts producer species, so, this species soon causes coccidiosis in chickens only a few weeks old, while *E. necatrix* is equally virulent but a poor oocysts producer; so it produces the disease in older birds.

The prevalence of coccidia infection primarily affected by the age of the chickens (Sharma *et al.,* 2015), the higher prevalence of coccidiosis at the age of 32–46 days might be associated with the presence of another immunosuppressive disease, such as Gumboro. Young birds are more susceptible and more readily display signs of disease, whereas older chickens are relatively resistant as a result of prior infection (Cervantes *et al.,* 2020).

The life stages of *Eimeria species* develops an endogenous intestinal phase within the host during which three or four rounds of asexual reproduction (schizogony) leading to formation of trophozoit, schizonts (I and II), merozoites (I and II), followed by sexual differentiation into micro and macrogamets (gametogony), fertilization and finally, shedding of the (unsporulated oocysts) within the excreta of the chicken (Francia and Striepen, 2014). Under suitable conditions (25–30°C temperature, moisture, and oxygen) and within 48–72 hours, the process of sporulation begins immediately, yielding sporocysts and infective sporozoites and become infective to another host (sporulated oocysts) (Fanatico, 2006).

Eimeria spp causing damage to the cells lining the intestines as it lives and multiply in the cells of the intestinal tract (Arabkhazaeli *et al.,* 2013). The severity of an infection depends on; the age of chicken, *Eimeria species*, number of infective stages that ingested, immune status of the flock and environmental management. Infected birds tend to gathered together, have depression and ruffled feathers. Certain species of *Eimeria* cause dysentery, enteritis, diarrhea, which may be bloody. Emaciation, feed conversion is in lower rate, delayed sexual maturity, drooping wings lead to dehydration and weight loss as well as mortalities. (Abbas *et al.,* 2017).

This study aimed to detect the prevalence of *Eimeria species* in chicken and detection of its pathological effect within the intestinal mucosa and submucosa.

MATERIALS AND METHODS

The present study was carried out during the period from August 2020 to August 2021 in the department of Parasitology, Faculty of veterinary Medicine Assiut University.

Study flocks and samples

The study was conducted on 150 (intestinal gut samples) taken from broiler-chicken that obtained from different chicken farms and diagnostic labs in Sohage Governorate, Egypt. After birds' necropsy, the intestine of each bird was dissected out; divided into small intestine and large intestine, then evacuated and scrabed separately into respectively labeled plastic cups, sieved, and preserved using potassium dichromate 2.5 % (in ratio of 1:3) and neutral buffer formaline 10%. Muciod specimens were mixed with several drops of 1% of KOH to avoid trapping of protozoan oocysts during sieving (Gracia, 2001).

Gross examination

Intestinal wall, mucosa and serosa were examined for thickening, other pathological changes as haemorrhage, congestion, corregations, tissue debris white spots, and ulceration. Also, blood and abnormal content

were examined if present within the intestinal contents grossly (Garcia, 2001).

Parasitological examination:

a. Direct examination: through

1- **Unstained wet mount technique (Garcia, 2001):** a drop of the scraped intestinal mucosa put on clean slide and mixed with a drop of 0.9% saline, thoroughly mixed till forming uniform smear. For detection of any coccidian oocysts

2- **Concentration technique:** Positive *Eimeria spp*. samples were concentrated using saturated salt floatation concentration technique according to (Cringoli *et al.*, 2010).

b) Sporulation of coccidian oocysts according to (Rao *et al.*, 2013)

For perfect identification, the coccidian oocysts were sporulated. In clean glass petri dishes, the positive faecal samples for *Eimeria species* were mixed with 2.5% potassium dichromate solution at the depth of 3-5 mm. Petri dishes. The covers of the petri-dishes were lined by moist filter paper and left to stand at room temperature. They were daily aerated and examined to follow up the process of sporulation. The contents of these petri-dishes were concentrated by floatation technique, for identification of the morphological characters of sporulated *Eimeria* oocysts.

c- Histopathological examination acc. to **(Bancroft *et al*., 1996)**

Intestinal specimens were taken from infected chickens, fixed in 10% formaline, dehydrated, cleared, and then embedded in paraffin blocks. 5 µm thicknesses were taken from Paraffin sections, stained by haematoxylin and eosin and examined microscopically.

Statistical analysis

The collected data were analyzed by Statistical Package for Social Sciences v.20 for Windows (SPSS). The significance of differences between the groups were calculated using the Chi-square test for trend analysis (p-value of < 0.001 considered significant).

Ethical consideration

The animal studies were conducted in accordance with the international valid guide lines.

RESULTS

Our study revealed that about 99 birds were infected with *Eimeria species* from 150 parasitologically examined diseased chickens, with a prevalence rate of 66%. The incidence rate in Broiler chickens was 70% (70/100) and in Balady was 58% (29/50) as shown in Table.1. The examined birds had a history of diarrhea, uneven growth, and bad feed conversion. The post mortem examination revealed enteritis at different localities of the intestine.

In relation to effect of age on the prevalence of infection, there is no infection with Eimeria sp. from 0 to 15 day. The highest percent of infection was at the age of (15-30) day (54.3% in Broiler and 72.4% for Balady), followed by (45.7% in Broiler and 17.2% for Balady) at the age of 30-45day while the lowest infection was detected at the age of higher than 45 day (10.34% in Balady and no infection for Broiler) as shown in Table (2) .

In relation to seasonal prevelance of *Eimeria spp*, the prevelance of Eimeria sp was highest in winter. The percent of infection was (88.5% in broiler and 75% for Balady), followed by autumn (82.6% in broiler and 53.8% for Balady)}, while in summer was (54.1% in Broiler and 54.5% for Balady), finally, in spring, the percent of infection was (55.5% in Broiler and 50% for Balady) as shown in (Table 3).

In relation to Mixed infection (infection with more than one species of *Eimeria*) only about (6/29 from Balady and 16/70 from Broiler were infected with more than one

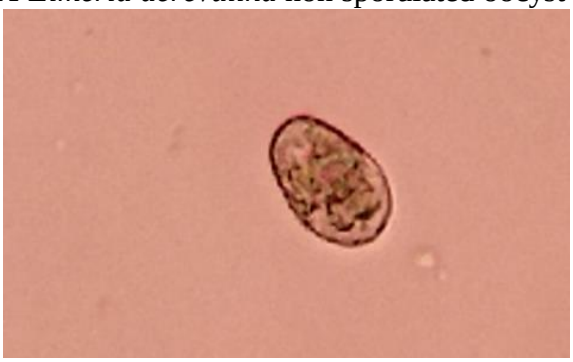
type of *Eimeria*. the percent of infection is 20.7% in Balady and 22.86% in Broiler).while the rest of examined infected chicken were infected with one species of *Eimeria*. (23/29 from balady and 54/70, the percent of infection is 79.3% in Balady and 77.14% in Broiler) as shown in (Table 4).

The species that were detected are *E.acovullina* (Fig.1) the highest prevalence rate followed by *E.tenella* (Fig.2) followed by *E.necatrix*, (Fig.3) followed by *E.mitis* (lowest prevelance rate) (Fig.4). The species of *Eimeria* were identified according to shape, size and sporulation time as shown in figures.

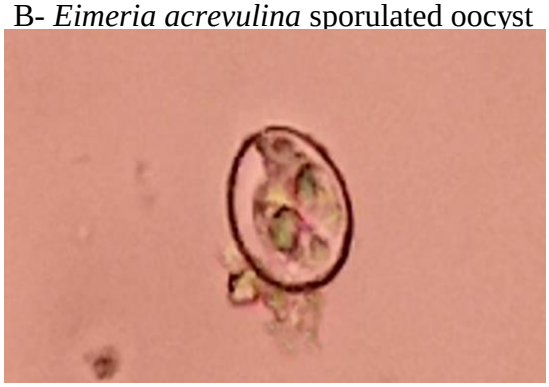
Histopathological examination:

In relation to the pathological changes in the intestinal mucosa, the intestinal epithelial cells showing different changes, there was severe necrosis of intestinal villi and presence of necrotic tissue in the intestinal lumen with inflammatory cell reaction at the intestinal mucosa with different stages of *Eimeria sp* (trophozoites, schizont and oocysts) associated with heavy cellular infiltration at the mucosa and sub mucosal tissues. As shown in the figure (5 and 6).

A-*Eimeria acrevulina* non sporulated oocyst

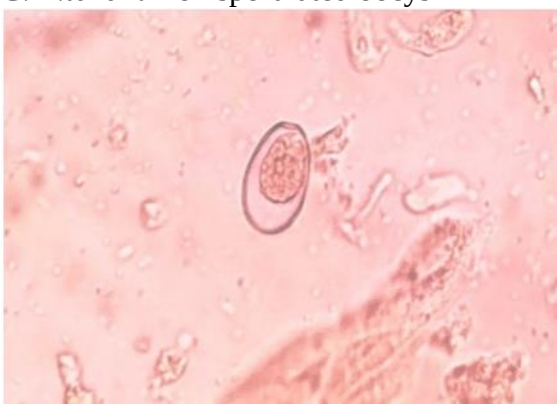


B- *Eimeria acrevulina* sporulated oocyst

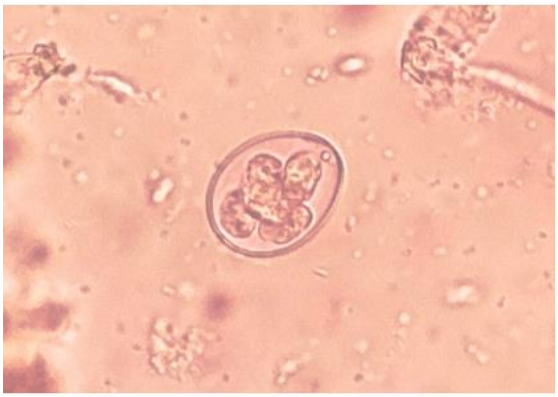


(Fig.1) Showing *Eimeria acrevulina* X400 (that are usually oblong oval with two layered wall), Average size: 18 x 15 µ. Sporulation time: 17 h.

C. *E.tenella* non sporulated oocys

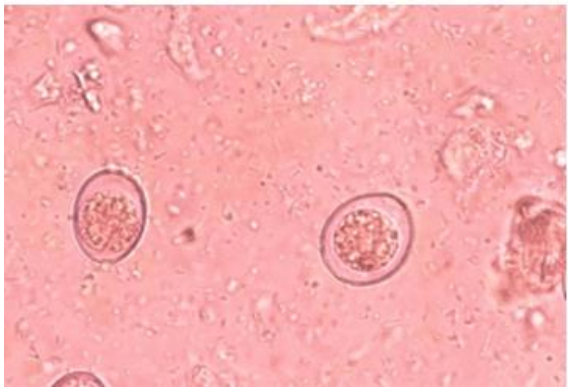


D. *E.tenella* sporulated oocyst

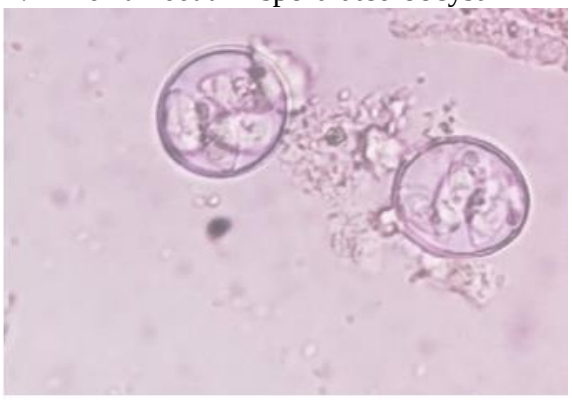


(Fig.2) Showing *E.tenella* X400 (that are usually ovoid with smooth two layered wall), Average size: 22 x 19 µ. Sporulation time: 1 day.

E. *Eimeria necatrix* non sporulated oocyst

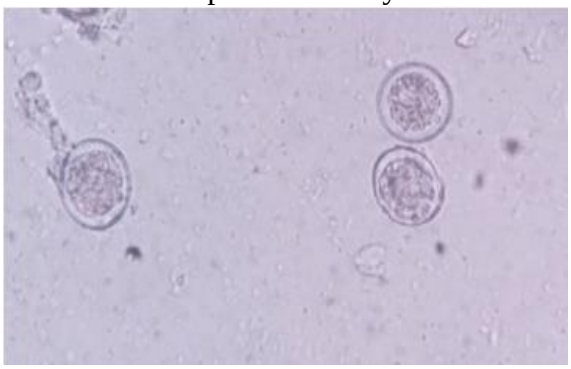


F. *Eimeria necatrix* sporulated oocyst

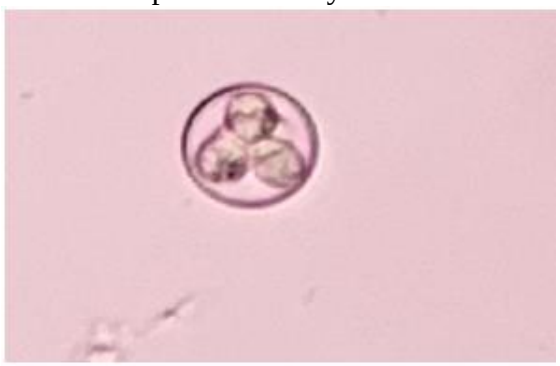


(Fig.3) showing *Eimeria necatrix* X400 (are usually oblong ovoid with smooth wall without micropyle), Average size: 20 x 17 µ. Sporulation time: 19 h.

G- *E.mitis* non sporulated oocyst



H- *E.mitis* sporulated oocyst



(Fig.4) Showing *E.mitis* X400 (are usually sub-spherical with smooth wall without micropyle), Average size: 17x 14 µ. Sporulation time: 20h.

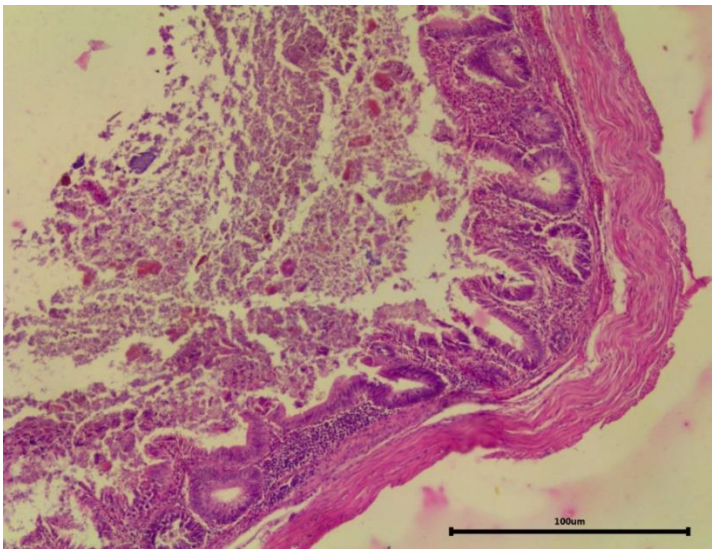


Fig. 5: Photo micrograph of intestine from chicken (Balady) showing sever necrosis of intestinal villi and presence of necrotic tissue in the intestinal lumen X100.

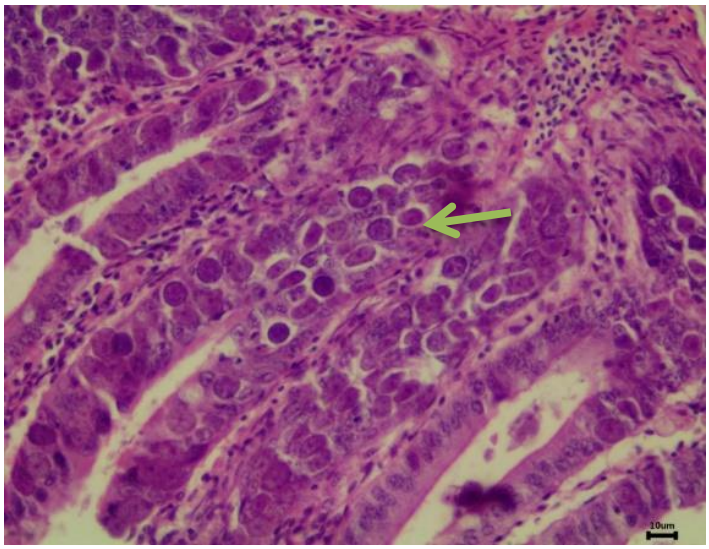


Fig. 6: photo micrograph of intestine from chicken (balady) showing different stages of coccidian parasite associated with hemorrhage and inflammatory cell infiltration X400. The arrow directed to the different stages of Eimeria spp.

Table 1: Total prevelance of *Eimeria spp* in both Broiler and Balady.

Total prevalence	*Eimeria* ssp	*P* value
Broiler	70 (70/100)	<0.0001***
Balady	58 (29/50)	0.12

No. /T= number of infected /total number of examined birds
Chi-square for trend analysis was used to compare between the proportion of infection in balady and broiler.
Significant difference in broiler group (P< 0.0001.)

Table 2: Prevelance of *Eimeria spp* according age amonge balady and broiler.

Age	0-15day	15-30day	30-45day	> 45day	*P* value
Broiler (No./T)	0/70 (0)	38/70 (54.3)	32/70 (45.7)	0/70 (0)	<0.0001***
Balady (No./T)	0/29(0)	21/29 (72.4)	5/29 (17.2)	3/29(10.34)	<0.0001***
Total (No./T)	0/99 (0)	59/99 (59.6)	37/99(37.4)	3/99 (3.03)	

Chi-square for trend analysis was used to compare the proportion of infection in related to the age
Significant difference between different age group P< 0.0001.

Table 3: Sesonal prevalence of *Eimeria* spp.

Seasonal Prevalence	Broiler	Balady	Total (broiler+balady)	*P* Value
	No./T (%)	No./T (%)	No./T (%)	
Winter	23/26 (88.5%)	9/12 (75%)	84.4%	0.013*
Spring	15/27 (55.5%)	7/14 (50%)	53..66%	0.088
Summer	13/24 (54.1%)	6/11 (54.5%)	54.3%	0.108
Autumn	19/23 (82.6%)	7/13 (53.8%)	72.2%	0.019*
Total	70/100	29/50	99/150 (66%)	<0.0001***
***P* value**	0.02*	0.055		

Chi-square for trend analysis was used to compare the proportion of infection in related to the season.
Significant difference between total infection in broiler and balady groups in related to season (P< 0.0001).

Table 4: Single and mixed infection among Balady and Boiler.

	Single *Eimeria* infection		Mixed *Eimeria* infection		Total	*P* value
	N	%	N	%	N	
Balady	23	79.31	6	20.7	29	<0.0001***
Broiler	54	77.14	16	22.86	70	<0.0001***

Chi-square for trend analysis was used to compare single and mixed infection of infected birds.
Significant difference between single and mixed infection in both Broiler and Balady (P< 0.0001).

DISCUSSION

Coccidiosis is worldwide disease problem of intensively reared chickens. Researchers, veterinarians and economists are interested in coccidiosis over many years, due to its severe economic losses among the infected birds, particularly poultry industry where coccidiosis is the most problematic than other lifestocks. In Egypt, poultry production appears to be the most important of farm animal (FAO, 2006).

The present study revealed that about 99 birds were infected with *Eimeria species* with a prevalence rate 66%. The incidence rate in Broiler chickens was 70% and in Balady was 58% in relation to the total number examined. The examined birds were diseases and had one or more health problem. The infected birds showed many signs (including dullness, uneven growth, decreased body weight, ruffling and mortality). This incidience of *Eimeria species* infection among the diseased chickens was indicative to endemicity of the coccidiosis among chickens. Also it gives an idea about inresponsivity of the prevalent *Eimeria species* in field to the used protective anti-coccidials in feed and also indication to bad mangemental measures.

Our results nearly similar to (Gari *et al.*, 2008) who showed that the prevelance rate of *Eimeria* was (61.25%) and (Olanrewaju and Agbor 2014) (69%) while it more than that recorded by (Oljira *et al.*, 2012) (20.57%) and (Garbi *et al.*, 2015) (19.5%). While our results where low when compared to (Dinka and Tolossa 2012) in Ethiopia (71.7%), (Al-Quraishy *et al.*, 2009) in Saudi Arabia (80%) and (Lawal *et al.*, 2016) in Nigeria (87.4%). In this study balady chickens was 58%, this result unlike (Ahmed *et al.*, 2003) and (Amer *et al.*, 2010) in Egypt, who recorded the rate of infection (43.9 %) and (90%) respectively.

In this study Broiler breeds had high coccidian infection rate (70%). This might be connected to higher stocking densities in broiler production in the study area. The high prevalence also might be due to several factors such as high humidity, time of sampling, poor managment and the environment such as accumulation of feces that support the development of *Eimeria* oocysts.

In relation to effect of age on the prevalence of infection, from 0 to 15 day there is no infection with *Eimeria sp*. This study agree with studies made by (Etuk *et al.*, 2004), (Amare *et al.*, 2012), and (Dakpogan and Salifou 2013) and disagree with (Badran and Lukešová 2006; Sharma *et al.*, 2015) and (Omer *et al.*, 2011). Absence of infection in age from 0 -15 days this return to protection by maternal imunity, GIT of bird in efficient to crush and digest the oocysts, also, young bird unable to take sufficient number of oocysts to produce infection.

The highest percent of infection was at the age of (15-30) day (54.3% in Broiler and 72.4% for Balady), this nearly agree with (Razmi and Kalideri 2000), (Shirzad *et al*., 2011), (Oljira *et al.,* 2012) and (Muazu *et al.,* 2008) and disagree with (Sharma *et al.,* 2015). *Eimeria spp*. can caused infection in all ages of poultry. The age of the chickens is considered as a very important factor in the prevalence of coccidiosis infection. (Badran & Lukesouna 2006; Sharma *et al.,* 2015). Higher prevalence of coccidiosis at the age of 30–46 days might be associated with the presence of another immunosuppressive disease, such as Gumboro (Hachimi *et al.,* 2008; Lanckriert *et al.,* 2010; McDougald & Steve 2008).

In the present study seasonal prevelance showed that the highest infection rate *Eimeria spp* was in winter season (88.5% in Broiler and 75% for Balady), followed by autumn (82.6% in Broiler and 53.8% for Balady), while in summer (54.1% in broiler and 54.5% for Balady), finally, in spring (55.5% in Broiler and 50% for Balady), these results were agree with (Shirley, 1992) and (Ashenafi *et al.,* 2004) who explained that the effect of humidity increase the percent of infection in winter. Also, increasing incidence of coccidiosis in winter is due to increasing the stocking density in winter which may reach to 30% (Lunnden and Thebo 2000) and (Badawy *et al.,* 2000).

Our study was detected that there were different species of *Eimeria* foud within the same bird (Mixed infections) this result agree with (Haug *et al.,* 2008) and (Aarthi *et al.,* 2010), who detected that multiple infections with two or more *Eimeria spp*. were observed in some of the positive cases. In this study, single infection was observed more than mixed infection this parallel to study in Romania (Haug *et al.,* 2008).

The histopathological examination showed different changes (sever necrosis of intestinal villi and presence of necrotic tissue in the intestinal lumen, with inflamartory cell reaction at the intestinal mucosa with different stages of Eimeria sp (trophozoites, schizont and oocysts) this in agreement with (Zyan *et al.,* 2017).

In Conclusion, This study indicate that the coccidiosis is a serious parasitic disease that affect the poultry production in Egypt and control measures should be put in consideration to overcome this disease.

ACKNOWLEDGMENTS

We would like to thank Prof. Dr. Sary Khaliel Naser, Professor and head of pathology Department, faculty of veterinary medicine for his help in histopathological examination. Also we want to thank Dr. Sayed kalaf and Dr. Naglaa Fathy for their help in collection of samples.

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مدى انتشار الكوكسيديا في الدجاج بمحافظة سوهاج

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تعتبرالايميريا من أهم الطفيليات التي تسبب خسائر اقتصادية عالية جدا في مزارع الدواجن في مصر. وتسبب مرض يسمى الكوكسيديا. هدفت هذه الدراسة إلى الكشف عن انتشار أنواع الايميريا المختلفه في الدجاج والكشف عن الايميريا وتأثيرها المرضي داخل الغشاء المخاطي المعوي. وتبحث الدراسة الحالية عن انتشارهذا المرض عن طريق. فحص البراز واستند التشخيص إلى فحص عينة البراز بالطرق المباشره وكذلك بتقنيات التركيز لزياده عدد الطفيل ، متبوعًا ببلوغ الطور المتحور لتحديد نوع الايميريا. وأخيرا دراسة التأثير المرضي لهذا الطفيل على الغشاء المخاطي المعوي للدجاج المصاب. واوضحت الدراسه ان معدل الانتشار الإجمالي لـ لايميريا بلغ (٦٦٪) وبلغت نسبة الإصابة في دجاج التسمين (٧٠٪) وفي البلدي (٥٨٪). وكانت أعلى نسبة إصابة في سن (١٥-٣٠) يوم ، وكان المرض أكثر انتشارًا في الشتاء عنه في الصيف. تشير هذه النتائج إلى أن الكوكسيديا مرض طفيلي خطير يؤثر على إنتاج الدواجن في مصر ويجب مراعاة تدابير المكافحة للتغلب على هذا المرض.