



Prevalence and morphological identification of *Eimeria* spp. in domestic rabbit (*Oryctolagus cuniculus*) in Sharkia province, Egypt

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ABSTRACT

Coccidiosis is widespread and common enteric protozoal disease of rabbits. The current study showed prevalence of *Eimeria* spp. amongst rabbits was 34.93%. Additionally, eight *Eimeria* spp. were identified using light microscope; whereas *E. coecicola* and *E. irrisidua* were more frequent among all *Eimeria* spp. while, four *Eimeria* spp. were detected using PCR methods. Further, rabbits with mixed infections (72.55%) of different *Eimeria* spp. were higher than those with single infections (27.45%). Histopathology also revealed a developmental stages of *Eimeria* in enterocytes of intestinal villi and desquamation of the epithelial lining the lumen. It can be concluded that the current research provides appropriate data that help for assessing the potential infection and future control measurements against rabbit coccidiosis to mitigate the financial losses in rabbit industry in Egypt.

Key words: *Eimeria*, Rabbits, Prevalence, Sharkia, Egypt.

INTRODUCTION

Eimeria is intracellular parasites that invade the intestinal and hepatic epithelial cells causing severe digestive conditions in rabbits (Cere et al., 1996; Tao et al., 2017). Hence, rabbit industry is well developed as it provides a good source of a healthy meat and highly demand in Egypt as well as rabbits characterized by high growth rate with good food utilization (Lebdah and

Shahn, 2011; Emam et al., 2020).

Therefore, coccidiosis evoked major economic losses for rabbit production industry especially of lower-income families; even sub-clinical health animals suffer from low food intake, decreased feeding conversion and retarded growth rate (Yin et al., 2016; El-Ashram et al., 2020). Update, eleven *Eimeria* spp. infect rabbits were identified wherever morphometry of

the oocysts and pathogenicity were more distinct between species (**Kvicerova et al., 2008**). However, ten of *Eimeria spp.* which include *E. piriformis*, *E. perforans*, *E. media*, *E. vej dovskyi*, *E. magna*, *E. coecicola*, *E. flavescens*, *E. intestinalis*, *E. irresidua* and *E. exigua* which localized in different sites of rabbit intestinal tract (**Pakandl, 2009**); thereby causing intestinal cells disruption and electrolytes imbalance with poor growth and metabolism (**Metwaly et al., 2013**). While, only one species of hepatic coccidiosis; *E. stiedae*, which parasitize exclusively the rabbit liver and the biliary tract causing hyperplasia of epithelial of the bile duct with the developing stages (**Darzi et al., 2007; Sivajothi et al., 2016**). *Eimeria* oocysts are passed in fecal samples of infected rabbits to environment causing infection to rabbits with both intestinal and hepatic forms through ingestion sporulated oocysts with contaminated food and water (**Hamid et al., 2019**).

Several studies of rabbit coccidiosis worldwide has been reported which the prevalence was 56.4 % in China (**Yin et al., 2016**). While, **Okumu et al. (2014)** indicated the prevalence of *E. stiedae* and intestinal coccidiosis was 11.5% and 29.5% respectively in Kenya. Accordingly, *Eimeria* oocysts in rabbits can be diagnosed mainly

by routine microscopy, but differentiation between all *Eimeria spp.* is difficult due to considerable variations among oocysts dimensions (**Yan et al., 2013**). Consequently, molecular PCR assays are required as a specific and sensitive tool for diagnosis and discrimination of *Eimeria spp.* that infect rabbits (**Cere et al., 1995; Oliveira et al., 2011**). Therefore, accurate *Eimeria spp.* identification is substantial to evaluate the purity of strains to develop a potential multivalent vaccine (**Oliveira et al., 2011**). In Egypt, the majority of studies were focused on histopathology and treatment particularly *E. stiedae*, yet, there has been limited research and scarce knowledge about *Eimeria* infections and biology in rabbits (**El-Shahawi et al., 2012**). Therefore, there is a little epidemiological data in respect of prevalence of rabbit coccidiosis in northern Egypt especially in Sharkia province which is characterized by different climatic and geographical conditions than in other provinces with the most of previous studies were conducted in Upper Egypt. Therefore, the objectives of the current work were to determine the prevalence of *Eimeria spp.* infections in rabbit's market shops in Sharkia province with associated risk factors of season, age and sex. Furthermore, morphology of

Eimeria spp. sporulated oocysts were described as well as PCR amplifications of ITS-1 region of *Eimeria* spp. were done. Additionally, histopathological lesions due to intestinal coccidiosis were described.

MATERIALS AND METHODS

Collection of fecal samples

A total of 146 of 20 grams of fresh fecal pellets were collected from naturally infected domestic rabbits (*Oryctolagus cuniculus*) in different poultry and rabbit selling market shops of Zagazig, Sharkia province, Egypt. The study included 77 males and 69 females. These rabbits were obtained from large and small holder farms and kept in individual cages which represent the common housing methods in market shops. Furthermore, fifty of livers and gall bladders were obtained from rabbit sellers upon legal regular animal slaughtering. Age of rabbits in the current study was considered in two groups of < 6 months and \geq 6 months. Clinical signs of animals were recorded. Each sample was kept in a labelled plastic bottle in ice boxes and transferred to Parasitology laboratory and stored at 4°C for examination. Samples were collected during a period ranged from November, 2016 and October, 2017.

Parasitological examinations

Fecal samples were examined by direct microscope smear and standard

concentration sheather's sucrose floatation methods were used to isolate *Eimeria* oocysts as described by You (2014). Hence, livers and gall bladders were checked for presence of *E. stiedae* oocysts through maceration of liver and sieving to remove coarse particle (AbouLaila et al., 2020).

The obtained washed oocysts were suspended in 2.5 % (w/v) aqueous potassium dichromate solution in partially covered petri plates at 25°C to 30°C for 7 days to induce sporulation and to ensure good oxygenation and examined periodically (Ryley et al., 1976). In each sample, sporulated oocysts were identified based on morphological characters (size, shape, presence or absence of the micropyle and residual) of oocysts and sporocysts (Al-Quraishy, 2012).

DNA Extraction

DNA was extracted from oocyst positive fecal samples using commercial kits (QIAamp DNA stool Mini Kit, QIAGEN, Hilden, Germany) following the manufacturer's protocols with some modifications. Briefly, fecal sample (220 mg) with adding 1.4 ml of ASL buffer and incubated at 70 °C for 5 min, homogenized for 6 min using tissue lyser, then centrifuged at 14,000 rpm for 1 min. One InhibitEx tablet added, vortexed and incubated for 1

min then centrifuged 14,000 rpm for 3 min. Proteinase Kinase (30 μ l) and lysis buffer (200 μ l) incubated at 70°C for 10 min. After incubation, 200 μ l of absolute ethanol was added following the manufacturer's recommendations. Isolated DNA was eluted in 50 μ l elution buffer and kept at – 20 °C for later analysis.

Molecular identification

ITS-1 gene amplified and primers designed by protocol provided by **Oliveira et al. (2011)**. PCR amplifications were performed. Using gel electrophoresis with 1.5% agarose gel in 1x TBE buffer at room temperature, PCR products were visualized.

Histopathological examination

Histopathological examinations were done to detect the developmental stages of *Eimeria spp.* in rabbits as well as pathological lesions within the intestine as described by **Kiernan (1981)**.

Statistical analysis

Data were analyzed with Chi square (χ^2) tests using IBM SPSS Statistics for windows software version 21. *P-values* <0.05 were considered statistically significant.

RESULTS

The current analysis showed the overall prevalence of *Eimeria spp.* in the examined rabbits was 34.93%. In addition, rabbits in the current study were apparently healthy,

showed no clinical signs. However, rabbits had a high infection rate with coccidiosis in both autumn (53.49%) and winter (39.02%) than other seasons with a statistical significance difference between four seasons and infection rate (Table 1). The present study showed that the prevalence of *Eimeria spp.* in animals less than six months (32.94%) was slightly lower than animals over six months (37.70%), with no statistical significant between two age groups. Meanwhile, the prevalences of *Eimeria spp.* in males and females were 31.17% and 39.13% respectively. Hence, there was no statistical significant between sex and infection rate (Table 2).

Based on morphology of sporulated oocysts, eight *Eimeria spp.* were identified which were *E. coecicola*, *E. perforans*, *E. media*, *E. exigua*, *E. intestinalis*, *E. flavescens*, *E. magna* and *E. irresidua* as shown in (Fig. 1 and Table 3). However, *E. coecicola* and *E. irresidua* were the most frequent *Eimeria spp.* which reached 56.86% and 43.14% respectively as well as concurrent infections of *E. coecicola*, *E. magna* and *E. irresidua* were more frequently (Fig. 3 and Table 4). However, no *E. stiedae* was detected either in the examined fecal pellets or liver and gall bladders. Furthermore, animals had a high percentage of mixed infections of

different *Eimeria* spp. (37/51) 72.55% than cases with single infections (14/51) 27.45% (Fig. 2).

Eimeria oocysts ITS-1 regions were amplified by PCR and analyzed by electrophoresis which four species were detected of *E. coecicola* (256 bp), *E. magna* (218 bp), *E. intestinalis* (269 bp) and *E. irresidua* (220 bp).

Histopathological findings revealed that the enterocytes of intestinal villi were with leukocytic infiltration in the lamina propria with partial mucosal desquamation. Furthermore, different *Eimeria* developmental stages were found in intestinal crypts and villi (Fig. 4).

Table 1: Prevalence of *Eimeria* spp. in rabbits at different seasons

Season	No. examined	No. infected	%
Autumn	43	23	53.49
Winter	41	16	39.02
Spring	29	7	24.14
Summer	33	5	15.15
Total	146	51	34.93

Table 2: Prevalence of *Eimeria* spp. in rabbits regarding different age groups and sex

Age groups	No. examined	No. infected	%
< 6 Months	85	28	32.94
≥ 6 Months	61	23	37.70
Total	146	51	34.93
Sex	No. examined	No. infected	%
Males	77	24	31.17
Females	69	27	39.13
Total	146	51	34.93

Table 3: Morphological characters of sporulated oocysts of *Eimeria spp.* of rabbits

<i>Eimeria spp.</i>	Oocyst					Sporocyst			Sporulation time (h)
	Shape	Length (µm)	Width (µm)	Micropyle	Oocystic residuum	Length (µm)	Width (µm)	Sporocystic residuum	
<i>E. exigua</i>	Spheroidal	14.64	13.57	Absence	Absence	6.67	4.57	Absence	20
<i>E. perforans</i>	Ovoid or ellipsoidal	18.36	12.65	Absence	Present	9.87	6.02	Absence	22
<i>E. flavescens</i>	Ovoid	27.83	19.44	Present	Absence	13.91	10.38	Present	51
<i>E. intestinalis</i>	Pyriiform	25.71	18.60	Present	Present	11.42	8.62	Absence	58
<i>E. coecicola</i>	Elongated ellipsoidal	28.10	15.64	Present	Present	11.76	6.30	Present	60
<i>E. media</i>	Ovoidal to ellipsoidal	27.48	17.79	Present	Present	10.60	6.42	Absence	34
<i>E. magna</i>	Ovoid	28.41	19.23	Present	Present	7.34	4.62	Present	43
<i>E. irresidua</i>	Ellipsoidal, with blunt end	29.87	19.57	Present	Present	15.10	9.83	Present	50

Table 4: Percentage of infected fecal samples related to each *Eimeria spp.*

<i>Eimeria spp.</i>	No. of Positive fecal samples	Prevalence (%)
	Domestic Rabbits (n=51)	
<i>E. coecicola</i>	29	56.86
<i>E. irresidua</i>	22	43.14
<i>E. magna</i>	21	41.18
<i>E. flavescens</i>	16	31.37
<i>E. media</i>	14	27.45
<i>E. perforans</i>	8	15.69
<i>E. intestinalis</i>	6	11.76
<i>E. exigua</i>	6	11.76

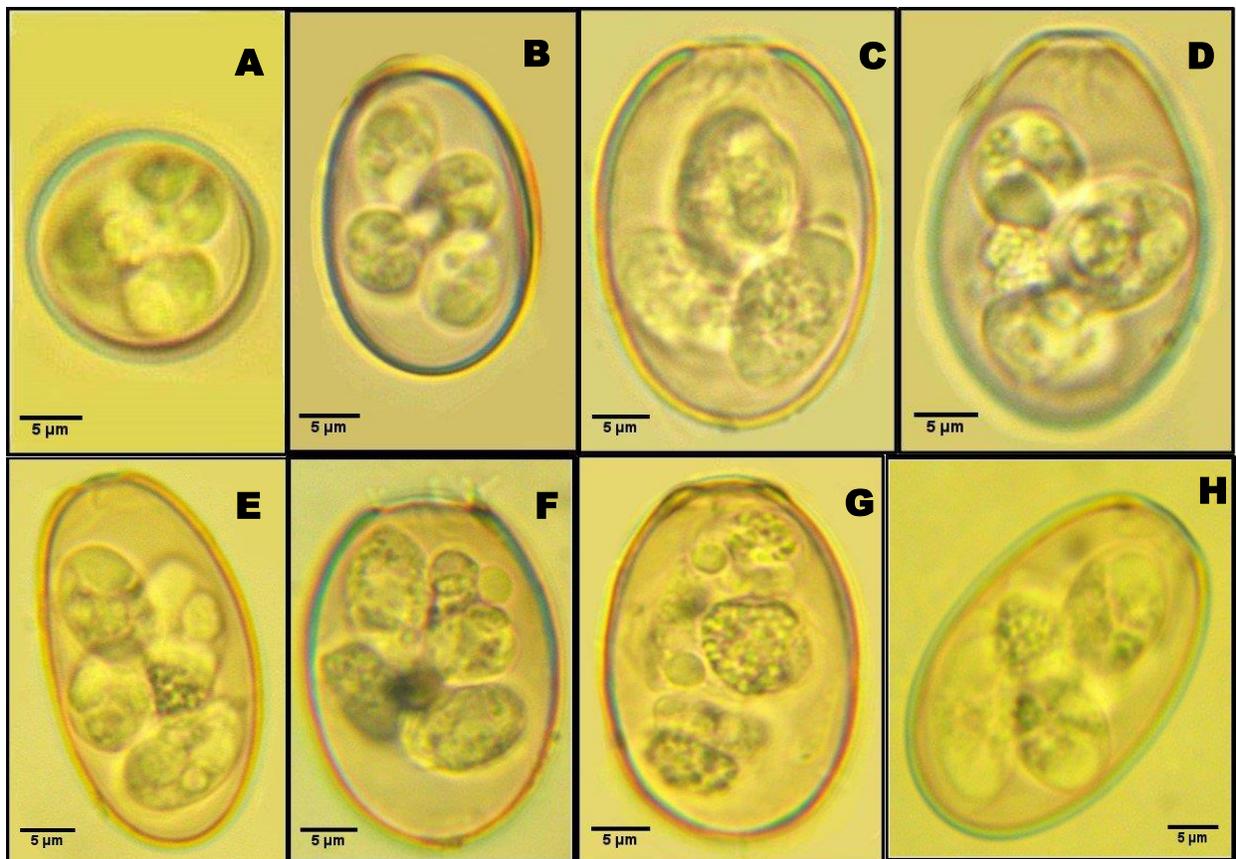


Fig. 1: Eight *Eimeria* spp. sporulated oocysts in rabbits. A. *E. exigua*, B. *E. perforans*, C. *E. flavescens*, D. *E. intestinalis*, E. *E. coecicola*, F. *E. media* G. *E. magna* H. *E. irresidua* (Bar= 5 µm).

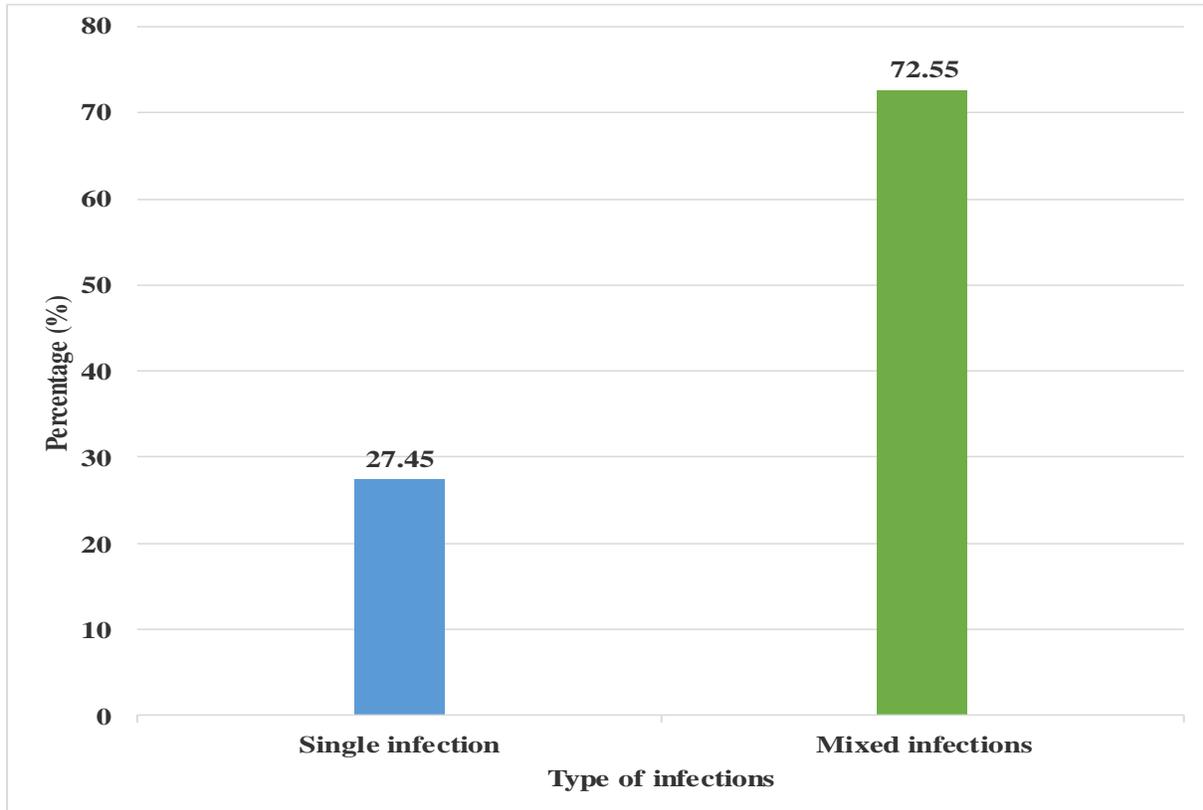


Fig. 2: Percentage of single and mixed infections among *Eimeria spp.* infections in rabbits.

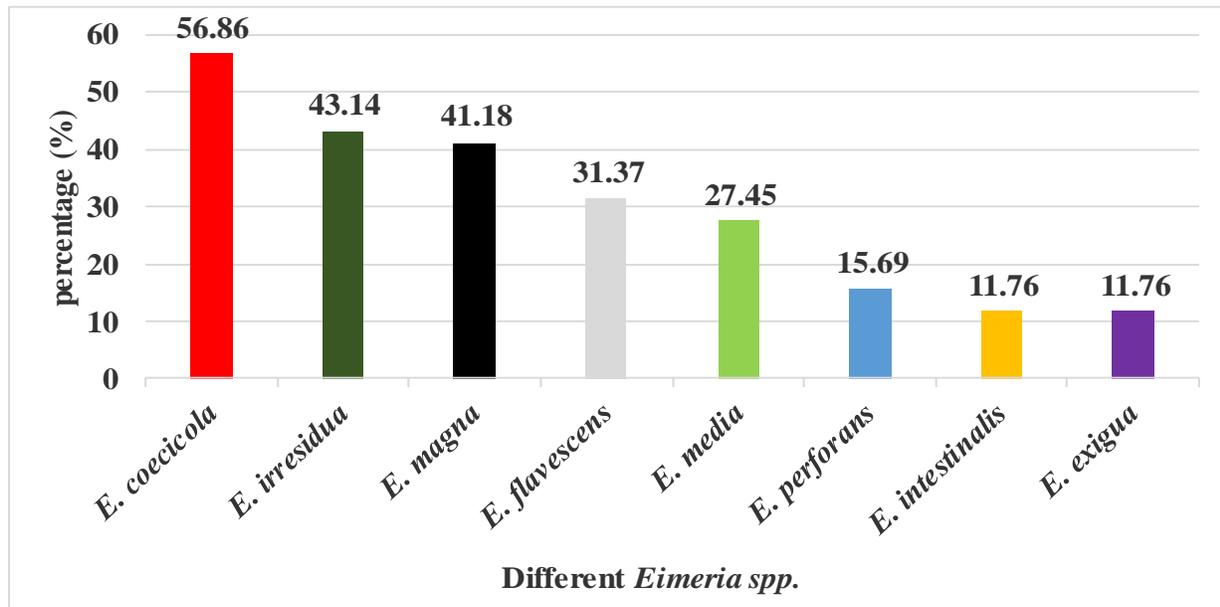


Fig. 3: The percentage of different *Eimeria spp.* in fecal pellets of rabbits.

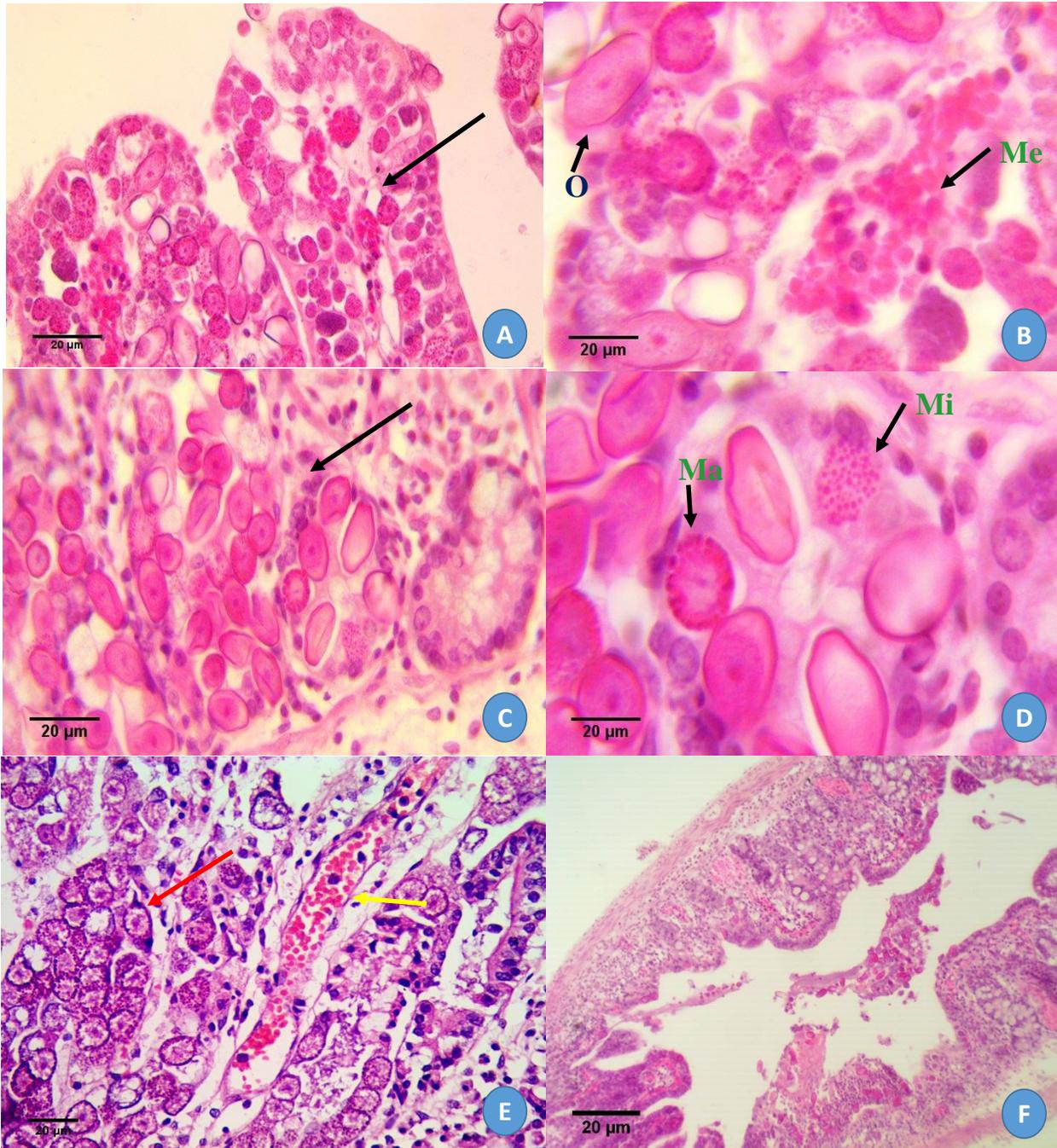


Fig. 4: Histopathological lesions of rabbit' intestine naturally infected with different *Eimeria* spp. **A, B, C, D.** Intestine of rabbit showing various developmental stages of *Eimeria* (**O**: Young oocysts; **Me**, Merozoites; **Mi**: Microgamonts; **Ma**: Macrogamonts) in the enterocytes of intestinal villi with leukocytic infiltration in the lamina propria, H&E, X40. **E.** Intestine of rabbit showing various developmental stages of *Eimeria* (red arrow) in the enterocytes of intestinal villi, with marked congestion and leukocytic infiltration in the lamina propria (yellow arrow), H&E, X40. **F.** Intestine of rabbit showing various developmental stages of *Eimeria* in the enterocytes of intestinal villi with leukocytic infiltration in the lamina propria and partial mucosal desquamation, H&E, X10. (Bar= 20 µm).

DISCUSSION

Rabbit coccidiosis has been recorded throughout the world causing severe economic losses, morbidity and mortality in rabbits (El-Akabawy et al., 2004; Hamid et al., 2019). Thus, the main purpose of the paper was to draw attention to update epidemiological information about the *Eimeria* infections in rabbits particularly in Sharkia governorate. As well, to our knowledge this is a recent study explores coccidiosis problems in rabbits in Sharkia province as only one study was done since many years ago by El Masry (1983).

In present study, the fecal analysis revealed that the prevalence of *Eimeria spp.* in domestic rabbits was 34.93%. The present findings were in good agreement with other studies such as Elshahawy and Elgoniemy (2018) who demonstrated that prevalence of *Eimeria spp.* in domestic rabbits was 33.9% in Upper Egypt. Unlike other literature studies, whereas our data was lower than recorded by El-Shahawi et al. (2012) and El-Ashram et al. (2020) in Beni Seuf, Egypt which prevalence rates reached 70% and 86.50% respectively. In addition, Hamid et al. (2019) and Maziz-Bettahar et al. (2018) recorded that 70.26% and 90% of Indonesian and Algerian rabbits were infected with coccidiosis respectively.

Moreover, Ming-Hsien et al. (2010) reported that rabbit coccidiosis of Taiwanese pet shops and farms were 46.2% and 41.7% respectively. While, our findings were higher than obtained by Chacko et al. (2017) in India (9.3%) and in Brazil (19.5%) by Heker et al. (2017). A difference between prevalence rates might attributed to various sampling methods, management systems, population density, different sampling methods, rabbit's breeds and feed as well as poor hygienic conditions (Jing et al., 2012; Heker et al., 2017; AbouLaila et al., 2020).

The present findings demonstrated that there was no a statistical significant between age and the prevalence rate. Although past researchers reported that animal age was an imperative factor and it has a significant effect on the latitude of the disease whereas high prevalence rates were recorded in young rabbits below 6 months (Pakandl, 2009; Pilarczyk et al., 2020). Additionally, a difference between the infection rates of young and old rabbits was reported by others as Chacko et al. (2017) who found a high prevalence of coccidiosis among rabbits of 1.5-2.5 month, but animals under 1.5 month which cannot be infected. Also, Heker et al. (2017) who observed a

significance difference between young of 80 days (46.67 %) and adult rabbits (14.81%). The most likely interpretation was related to a discerned decrease in the immunity and resistance in young rabbits, otherwise, to less infected was attributable to the immunity acquired from previous infections at very small doses of infective oocysts that might produce a huge measure of protection to the animals (Stodart, 1968; Pakandl et al., 2008; Pilarczyk et al., 2020). In consequence, adult rabbits which recovered from coccidiosis produce acquire immunity, but still carriers (Kulisic et al., 2006). One possible limitation of the current research was that we have focused on rabbits in markets which the common age usually above two months. However, these findings are not conclusive. Despite of these limitations, this study can be as a first report shed a light on coccidiosis problems among rabbits at markets. While, the current research cannot preclude these explanations, it appears useful to illustrate concerns that may conflict with these findings.

Seasonally, the current study has highlighted that there were a statistical significance between seasons and the prevalence rate which autumn and winter were the highest among different seasons. This pattern of results are approximately similar with

Elshahawy and Elgoniemy, (2018) and Ladron de Guevara et al. (2019). However, Chacko et al. (2017) found no statistical difference among seasons but high coccidiosis was recorded during winter. These findings might be explained as rainy seasons with high humidity promoting the sporulation of oocysts and management systems as well as different types of diets during seasons (Laha et al., 2015; Elshahawy and Elgoniemy, 2018).

Regarding to sex, the present findings revealed that the prevalence of *Eimeria spp.* in females (39.13%) higher than males (31.17%) with no statistical significance. Those results were in line with previous studies done by Ming-Hsien et al. (2010) and Pilarczyk et al. (2020). Nevertheless, Heker et al. (2017) and Elshahawy and Elgoniemy (2018) claimed that the difference was significantly higher in females than males. It can be believed that females during pregnancy and lactation were subjected to stress and hormone changes, thereby lowering the resistance and increasing susceptibility to infections (Heker et al., 2017).

In this study, eight intestinal *Eimeria spp.* were identified and *E. coecicola* (56.86%) and *E. irresidua* (43.14%) were the most frequent among all *Eimeria spp.* However,

El Masry (1983) identified only five intestinal *Eimeria* spp. (*E. perforans*, *E. media*, *E. magna*, *E. irresidua* and *E. exigua*) in the same geographical region. Thus, to our knowledge, *E. coecicola*, *E. flavescens* and *E. intestinalis* were first recorded in Sharkia province, Egypt. While, in Beni Seuf governorate, Egypt, **El-Ashram et al. (2020)** detected as the same as seven intestinal *Eimeria* spp. which *E. media* was the most prevalent species but *E. irresidua* wasn't detected. In Saudi Arabia, **Toula and Ramadan (1998)** have ascertained five *Eimeria* spp. from domestic rabbits considering that *Eimeria perforans* (65%) and *E. magna* (45%) were the highest among *Eimeria* spp. In spite of ten *Eimeria* spp. were recorded by **Hamid et al. (2019)** in Indonesia in which rabbits were highly infected with *E. flavescens* and *E. coeciola*. Furthermore, the present results showed that rabbits were more frequent infected with mixed infections of *Eimeria* spp. than ones with single infection. This was a common finding as reported by **(Toula and Ramadan, 1998; Yakhchali and Tehrani, 2007; El-Shahawi et al., 2012)**. Although no *E. stiedae* was recovered from any of investigated rabbits, **AbouLaila et al. (2020)** who found that *E. stiedae* was in

12.5% of rabbits examined at Minoufiya governorate, Egypt.

Our present monitoring revealed that rabbits were apparently healthy and no clinical signs were recorded. These findings were consistent with the findings by **Razavi et al. (2010)** in wild rabbits. Establishing *Eimeria* infections in rabbits with no clinical signs based mainly on acquired mucosal immunity to intestinal coccidiosis after ingestion of low dose of contaminated oocysts for long period that protect further new infections and thereby prevent acute disease (**Razavi et al., 2010**). Also, the different pathogenicity between individual *Eimeria* spp. impacted disease clinical symptoms (**Pilarczyk et al., 2020**).

The morphology and measurements in this analysis were roughly similar to those outlined by **El-Shahawi et al. (2012)**. Also, sporulated oocysts differed slightly in some features than other previous studies described by **Kasim and Al-Shawa (1987)** and **Hobbs and Twigg (1998)**. On the other hand, histopathological findings indicated presence of developmental stages of *Eimeria* in enterocytes with desquamation of epithelial lining of intestinal lumen and leukocytic infiltration in lamina propria (**Elshahawy and Elgoniemy, 2018**).

Furthermore, four *Eimeria spp.* were detected by PCR methods, whilst, further work will implicate molecular sequence data. Moreover, in order to better understand the economic losses due to coccidiosis in rabbits, more research is still required in terms of factors as season, age, breeding and rearing establishments of the large and smallholder farms which rabbits always obtained for market shops.

CONCLUSION

It can be inferred from the current work that *Eimeria spp.* was prevalent among domestic rabbits in market shops in Sharkia province, Egypt. Thus, this research suggested the significant of coccidiosis in market shops and thereby rabbit's farms as regards management practices are concerned. Also, based on data obtained, it possible help to build future preventive strategies to enable us to improve hygiene as well as regulation of drug administration of anticoccidial therapy to avoid drug resistance.

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الملخص العربى

دراسات وبائية ووصفية لأنواع الأيميريا التى تصيب الأرانب الأليفة فى محافظة الشرقية بمصر

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تعتبر الكوكسيديا من الأمراض المعوية المنتشرة والشائعة في الأرانب الأليفة. حيث أظهرت الدراسة الحالية أن معدل الإصابة بالأنواع المختلفة بين الأرانب الأليفة هو 34.93%. بالإضافة إلى ذلك، كان هناك ثمانية من أنواع الأيميريا المختلفة تم التعرف عليها من خلال الفحص المجهرى الضوئى. وأشارت هذه الدراسة أن الأنواع *E. irrisidua* و *E. coecicola* كانت هى الأكثر شيوعا فى براز الأرانب. بينما، تم فى هذه الدراسة تشخيص أربعة أنواع للأيميريا باستخدام تقنية بادئيات البى سى آر (PCR). من ناحية أخرى، فإن الأرانب المصابة بأكثر من نوع من الأيميريا (72.55%) كانت أعلى من حالات العدوى بنوع واحد فقط (27.45%). كما وضح علم التشريح المرضي عن مراحل تطور الأيميريا فى الخلايا المعوية من الزغابات المعوية وتقرش البطانة الظهارية فى التجويف المعوى.

لقد بينت نتائج هذه الدراسة البيانات الضرورية التى تساعد فى تقييم العدوى المحتملة ووضع استراتيجيات المكافحة المستقبلية ضد كوكسيديا الأرانب لتقليل الخسائر الاقتصادية فى صناعة الأرانب فى مصر.