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RESEARCH ARTICLE

Prevalence and Morphological Identification of Eimeria Species in Sheep in Sharkia Governorate, Egypt.

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ABSTRACT

A total of 146 samples of sheep feces were collected from animals in Sharkia Governorate, Egypt to detect the occurrence and morphological features of Eimeria species. To detect Eimeria oocysts, the samples were microscopically inspected by direct smear and centrifugal flotation by Sheather's sugar method. Risk factors including season, age and sex were studied. The overall prevalence was 41.7% with higher incidence in winter (70%) than in summer (24.4%). The infection rate was greater in females (44%) than in males (33.3%) and in animals under a year old (72.7%) than animals aged one to two years (48%), and animals aged more than two years (20.6%). According to the morphological characteristics of the sporulated oocyst, ten species were recognized. The most often observed Eimeria species was E. bakuensis, accounting for (47.5%) of the total, followed by E. ovinialis at (9.8%), E. ahsata at (8.1%), E. granulosa at (6.5%), and E. faurei, E. weybridgensis, E. parva, E. pallida, and E. marsica, all of which were present at an equal proportion of (4.9%). The lowest prevalent species was E. crandallis, accounting for (3.2%). Considering our findings, which indicate that more than one third or nearly half of the sheep in Sharkia are infected with Eimeria, and that the season and age of the animals are significant risk factors for infection with coccidiosis, therefore, there is an urgent need for a plan to manage the disease in Egypt. Hence, this disease is a significant factor in the economic losses due to mortality, weight loss, and decreased production in animals and it is essential to have a comprehensive understanding of its life cycle to develop effective preventative measures.

Keywords: Eimeria, Morphology, Sheep, Risk factors, Sharkia.

Introduction

In Egypt, there are around 5.5 million heads of sheep [1], and they have a significant economic impact due to their ability to thrive and produce under poor environmental conditions while being raised on affordable feed [2]. It is stated that in small ruminants, *Eimeria* is the most prevalent and significant gastrointestinal parasite [3]. *Eimeria* is an obligatory parasite within cells that primarily infects the digestive system of animals and is known for its high degree of host specificity. Its life cycle is

completed within a single host [4, 5]. Coccidiosis is a severe disease with significant economic implications caused by *Eimeria* species. Sheep are known to be infected by fifteen species of *Eimeria* [6-8], The sheep's small and large intestine included fourteen species, whereas the abomasum contained one species (*E. gilruthi*) [9], while *E. ovinoidalis*, *E. crandallis*, and *E. ahsata* being reported as pathogenic species in sheep populations [10, 11]. Generally, *E. ovinoidalis* and *E. crandallis* can result in diarrhea due to decreased fluid absorption, but they may also induce a potent protective response without causing any symptoms. On the other hand, *E. granulosa*, *E. faurei*, *E. parva*, *E. pallida*, *E. ahsata*, *E. bakuensis*, and *E. punctata* can impede animal growth due to nutrient malabsorption [12]. Although the first reports of coccidiosis in sheep were published in 1879 [13], the disease mostly affects young lambs, causing severe clinical signs such as diarrhea, dehydration, delayed growth and development, and high mortality rates. This is due to the lack of effective immunity against primary infections, leading to large economic losses in the sheep industry. However, subclinical infections can also have a considerable economic impact due to weight loss and lower production, which is often observed in adult animals [10, 14, 15]. Following recovery from coccidiosis, animals are largely resistant to contracting diseases from the same species. However, immunity is not absolute as even extremely harmful species oocysts can be found in the excrement of otherwise healthy animals [16]. Determining the proper preventive measures requires knowledge of the disease's innate characteristics [3, 17]. Several reports from Egypt, such as those from Suez [18], Dakahlia [1], and Sharkia [10], described the prevalence of coccidiosis in sheep. The prevalence rate in Suez was (57.7%), while in Dakahlia was (68.4%). A study in Sharkia determined the morphology and prevalence of *Eimeria* species in sheep [10], but information on the epidemiology of *Eimeria* in Sharkia Governorate is scarce. Therefore, this study aimed to determine the prevalence of *Eimeria* species and their morphological characteristics.

Materials and Methods

Animal and sampling

A total of 146 samples of sheep feces were collected from apparently healthy animals in rural areas of Belbis and Minya El-Qamh in Sharkia Governorate, Egypt during a period extended from May 2023 to April 2024. These samples were obtained directly from the rectum using plastic disposable gloves and plastic containers, with each flock containing between 3 and 80 heads. The animals were divided into three age groups: less than one year, 1-2 years, and older than 2 years. The samples were labeled, and the age and sex of animals as well as the season were recorded. Thereafter, the samples were transported to the Parasitology Laboratory at the Faculty of Veterinary Medicine, Zagazig University, as well as the Microbiology and Parasitology Laboratory at the School of Veterinary Medicine, Badr University, where they were stored at 4°C until examination within 48 hours. All fieldwork conducted in this study adhered to the Guidelines for the care and use of laboratory animals in Egypt. Additionally, it was approved by Zagazig University Institutional Animal Care and Use Committee under the approval number of ZU-IACUC/2-F-235-2023.

Microscopic examination

The samples were analyzed using the direct wet smear method under a microscope at 10x and 40x magnifications and were further purified using Sheather's sugar centrifugal flotation technique, with the aim of detecting *Eimeria* oocysts [19-21]. To prepare fecal samples for analysis, they were initially diluted with water to eliminate any large particulate matter, followed by filtration through a metal sieve with pores measuring 4 mm in diameter. Approximately one teaspoon of feces was combined with a minimal quantity of Sheather's sugar solution, which has a specific gravity of 1.27, in a plastic cup, and the resulting mixture was then transferred to a 15-ml Falcon centrifuge tube. The tube cap was placed on the tube to prevent aerosol formation. The tube was then centrifuged at 3000 rpm for two minutes [2]. Following centrifugation, a small drop from the surface of the flotation medium was collected using a Pasteur pipet put on a glass slide, a cover slip was applied, and it was inspected under a microscope (10x and 40x objective lens). Also, the centrifuge tube was filled with flotation solution until the tube was nearly full and a coverslip was added to the tube, allowing the oocysts to attach to the coverslip. Then, the coverslip was removed and placed on a glass slid [22].

Sporulation and storage of Eimeria oocysts

The positive samples were filtered through metal sieves and mixed with 2.5% potassium dichromate solution to avoid overgrowth of fungi and bacteria that kill protozoa. They were then placed in petri dishes with a thin layer of 3-5 mm and exposed to daily aeration, as oxygen is essential for the process of sporulation, which is necessary for the initiation of meiosis in oocysts [5]. The samples were incubated at a temperature range of 24-33°C for 8 days [10, 23]. The samples were subsequently stored in a refrigerator at 4°C to preserve the integrity of the oocyst wall. Additionally, the samples were stored in 2% potassium dichromate at 4°C for up to six months, resulting in only a slight loss of viability. Also, unsporulated oocysts were stored for the same period, while sporocysts or sporozoites were preserved using liquid nitrogen for long-term storage [24].

Morphological identification of Eimeria oocysts

Following sporulation, the parameters utilized for the identification of *Eimeria* oocysts were contingent upon the morphological characteristics of the oocysts, such as their shape, size, color, wall thickness, sporulation time and presence or absence of the micropyle, and its cap. The aforementioned parameters were derived from previous studies [25-29].

Statistical analysis

The positive number to the total number of sheep examined was used to calculate the prevalence of *Eimeria* infections in sheep. The test of chi-square ($\chi 2$) with A *p*-value of less than 0.05 is considered statistically significant in statistical analysis conducted using SPSS version 21.0 (SPSS Inc., Chicago, IL).

Results

In the current study, different species of ovine *Eimeria* based on the morphology of the unsporulated oocysts were detected (Figure 1). Furthermore, sporulation process and measurement of the diameter of the oocyst were conducted using the open-source ImageJ software version 1.54 g, released in October 2023, to confirm the morphology and diagnosis.

Depending upon the morphometric data of the sporulated oocysts, ten *Eimeria* species were identified namely, *E. ahsata, E. bakuensis (ovina), E. faurei, E. granulosa, E. ovinoidalis, E. parva, E. crandallis, E. weybridgensis, E. marsica,* and *E. pallida* (Figure 2).

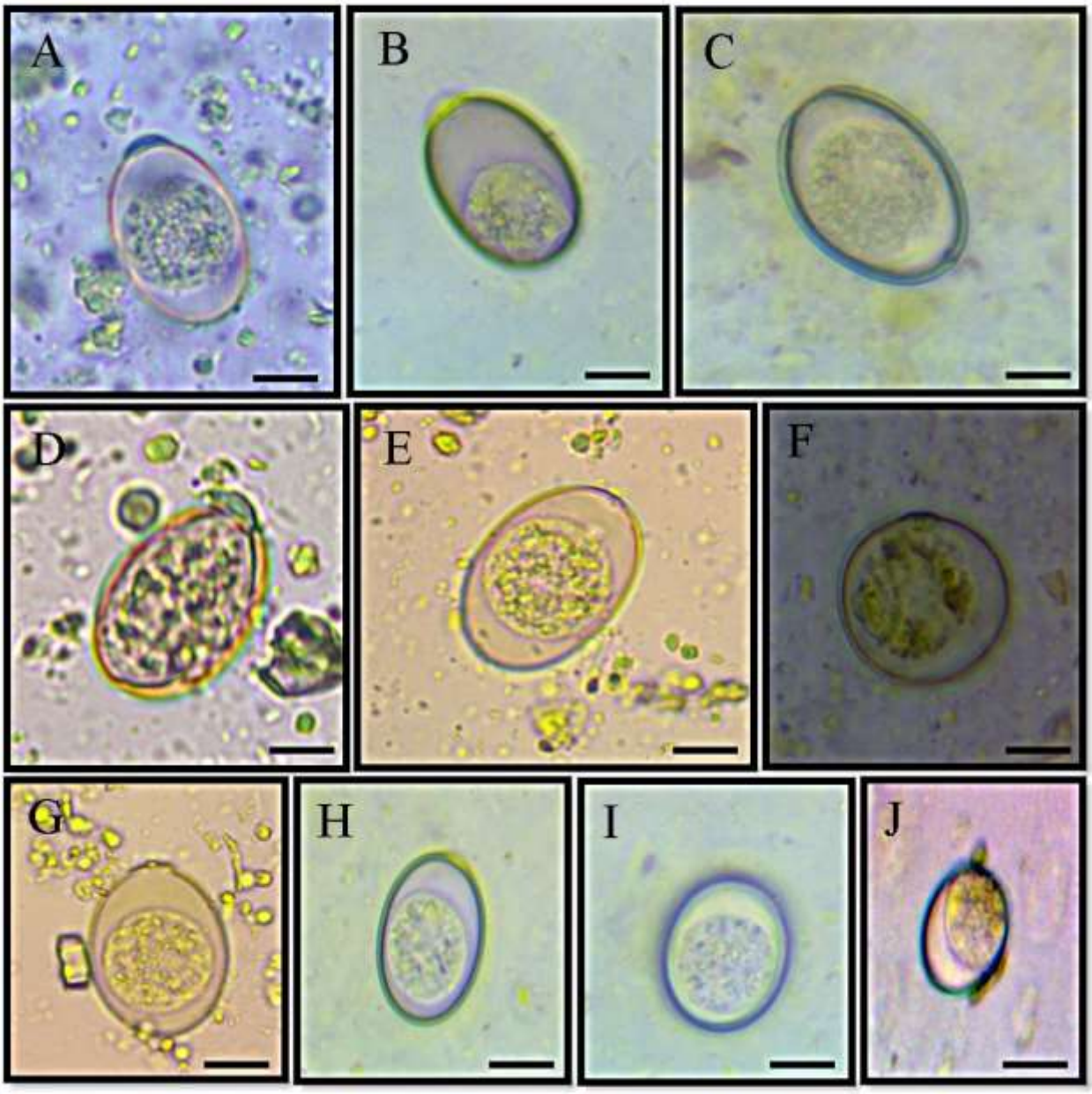

Figure 1: **Non-sporulated oocysts of *Eimeria* species in naturally infected sheep.**

(A) *E. ahsata,* **(B)** *E. bakuensis(ovina),* **(C)** *E. faurei,* **(D)** *E. granulosa*, **(E)** *E. ovinoidalis,* **(F)** *E. parva,* **(G)** *E. crandallis,* **(H)** *E. weybridgensis,* **(I)** *E. marsica,* **(J)** *E. pallida.* unstained. Scale bar 10 µm.

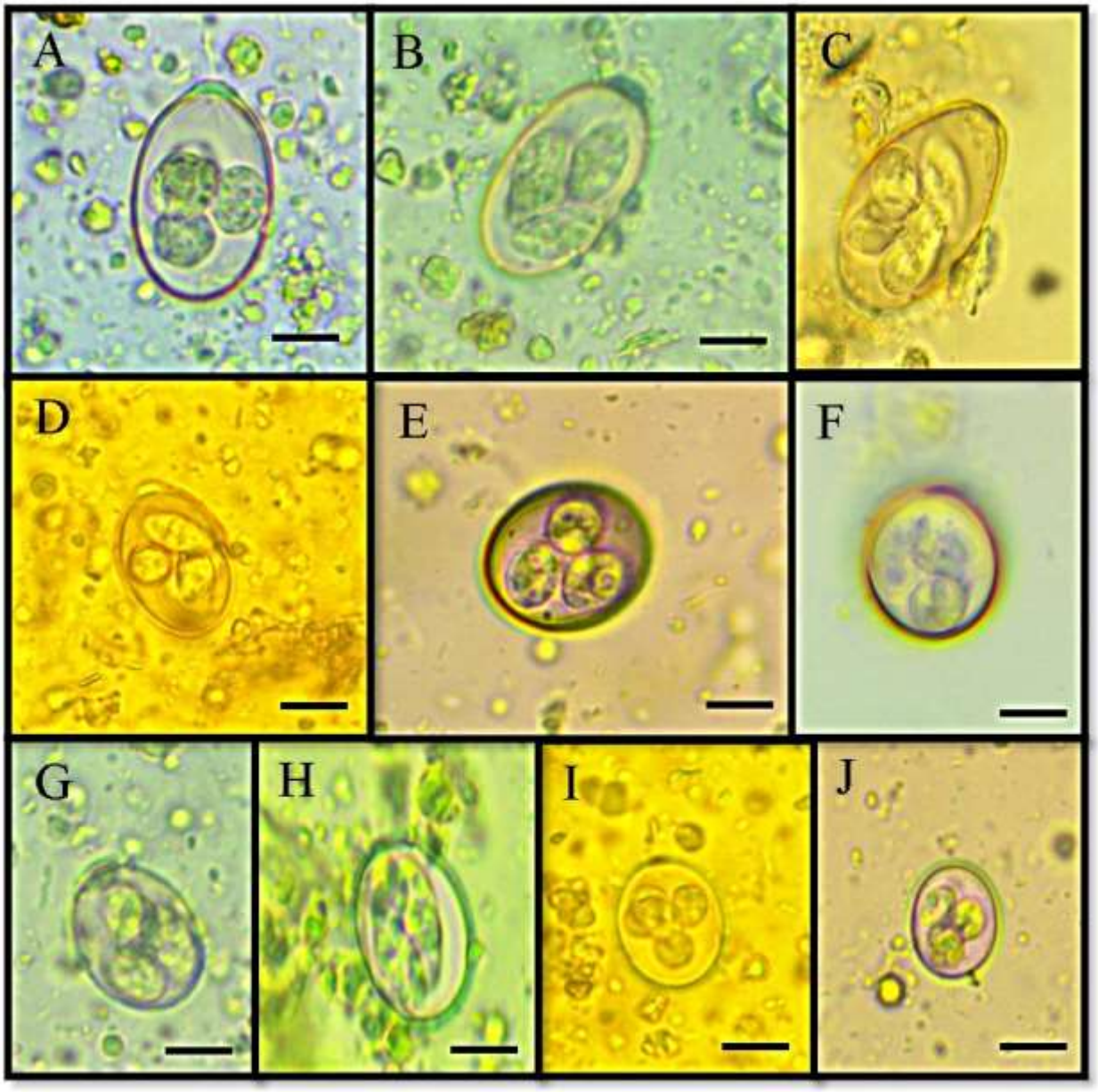

Figure 2: **Sporulated oocysts of *Eimeria* species in naturally infected sheep.**

(A) *E. ahsata,* **(B)** *E. bakuensis(ovina),* **(C)** *E. faurei,* **(D)** *E. granulosa,* **(E)** *E. ovinoidalis,* **(F)** *E. parva,* **(G)** *E. crandallis,* **(H)** *E. weybridgensis,* **(I)** *E. marsica,* **(J)** *E. pallida.* unstained. Scale bar 10 µm.

On the other hand, the sporulation time of *Eimeria* species was three days or less, *E. ahsata*, *E. bakuensis*, *E. faurei* and *E. granulosa* sporulated in three days, while the other identified species, *E. ovinoidalis*, *E. parva*, *E. crandallis*, *E. weybridgensis*, *E. marsica* and *E. pallida*, sporulated within two days. The results of the morphology and sporulation time for all *Eimeria* species oocyst are shown in Table 1.

Table 1. Morphological Characters of Sporulated Oocysts of Detected *Eimeria* spp. in the Examined Sheep at Sharkia Governorate

***Eimeria* spp.**		**Sporulated oocyst**					
		Shape	**Micropyle**	**Polar cap**	**Length**	**Width**	**Sporulation time**
E. ahsata	Fig. 2, A	ellipsoidal to ovoid	+	+ distinct	33.9 µm	20.8 µm	3 days
E. bakuensis (ovina)	Fig. 2, B	ovoid to Ellipsoidal (straight sides)	+	+ distinct	34.3 µm	18.16 µm	3 days
E. faurei	Fig. 2, C	egg-shaped	+	-ve	35.3 µm	20.9 µm	3 days
E. granulosa	Fig. 2, D	urn shape	+	distinct (easily dislodged)	31.2 µm	22 µm	3 days
E. ovinoidalis	Fig. 2, E	ovoid to ellipsoidal	barely seen	-ve	28.4 µm	22.79 µm	2 days
E. parva	Fig. 2, F	roundish	not seen	-ve	22.4 µm	20 µm	2 days
E. crandallis	Fig. 2, G	spherical to broadly ellipsoidal	+	+ may be absent	25 µm	18.6 µm	2 days
E. weybridgensis	Fig. 2, H	ellipsoidal to spherical	- ve	+ve	29.3 µm	17.5 µm	2 days
E. marsica	Fig. 2, I	ellipsoidal	inconspicuous	indistinct or absent	19.6 µm	15.7 µm	2 days
E. pallida	Fig. 2, J	ellipsoidal	not seen or imperceptible	-ve	17.5 µm	12.93 µm	2 days

Moreover, mixed infections were found in all examined fecal samples. Among the 146 apparently healthy sheep that were examined (116 from females and 30 males), no notable changes in color or odor were observed in the fecal samples. The ten *Eimeria* spp. that were identified based on the morphological characteristics of sporulated oocysts, were with a total infection rate of (41.7%; 61/146); the most common *Eimeria* species were *E. bakuensis* (47.54%; 29/61), *E. ovinoidalis* (9.84%; 6/61), *E. ahsata* (8.19%; 5/61), and *E. granulosa* (6.56%; 4/61), while *E. faurei*, *E. parva*, *E. weybridgensis*, *E. marsica*, and *E. pallida* were recorded in equal proportions (4.92%; 3/61), and the lowest prevalence was *E. crandallis* (3.28%; 2/61), as depicted in Table 2.

Table 2. Prevalence of Different *Eimeria* spp. Isolated from Naturally Infected Sheep.

Eimeria spp.	No. infected sheep	Prevalence %
E. ahsata	5	8.19 %
E. bakuensis (ovina)	29	47.54 %
E. faurei	3	4.92 %
E. granulosa	4	6.56 %
E. ovinoidalis	6	9.84 %
E. parva	3	4.92 %
E. crandallis	2	3.28 %
E. weybridgensis	3	4.92 %
E. marsica	3	4.92 %
E. pallida	3	4.92 %
Total	**61**	**100 %**

Additionally, with regard to sex and age, females had a higher infection rate (44%; 51/116) than males (33.3%; 10/30), animals younger than one-year-old had a high percentage of infection (72.7%; 24/33), followed by animals aged one to two years (48%; 24/50), and animals aged more than two years (20.6%; 13/61), as displayed in Table 3. Furthermore, infection in winter was (70%; 21/30) higher than in other seasons: fall (41.9%; 18/43), spring (39.3%; 11/28), and summer (24.4%; 11/45) (Table 3).

Table 3. Prevalence of *Eimeria* spp. according to Seasons, Sex and Age.

Risk factor		No. examined	No. infected	Prevalence %	Statistical analysis
Season	Winter	30	21	70 %	$\chi 2 = 15.453$ DF= 3 *P* value = .001 Significant (*P* <0.05)
	Fall	43	18	41.9 %	
	Spring	28	11	39.3 %	
	Summer	45	11	24.4 %	
Total		**146**	**61**	**41.7 %**	**-- -- -- -- -- -- -- -- -- -- -- -- -- --**
	< 1 Year	33	24	72.7 %	$\chi 2 = 25.369$ DF= 2 *P* value = .000

Age	≥ 1-2 Years	50	24	48 %	Significant (P <0.05)
	> 2 Years	63	13	20.6 %	
Total		**146**	**61**	**41.7 %**	-- -- -- -- -- -- -- -- -- -- -- -- -- --
Sex	Males	30	10	33.3 %	$\chi 2$ = 1.108 DF= 1
	Females	116	51	44 %	P value = .293 Non-Significant ($P \geq 0.05$)
Total		**146**	**61**	**41.7 %**	-- -- -- -- -- -- -- -- -- -- -- -- -- --

Discussion

The present study aimed to investigate sheep coccidial parasites in Sharkia Governorate, Egypt. Our findings revealed only 10 out of 14 species that were reported by Mohamed *et al.* [2] in Egypt. Additionally, Hassanen *et al.* [10] recorded *E. intricata* instead of *E. weybridgensis* in the same area. Morphological characters of different species of *Eimeria* agreed with that illustrated previously [25-29], sporulated oocyst of *E. ahsata* and *E. bakuensis (ovina)* are nearly similar but *E. bakuensis* has straight sides and sporocysts morphology is variable, oocyst of *E. weybridgensis* and *E. crandallis* are nearly similar but sporocysts morphology is variable [1]. The overall prevalence of *Eimeria* parasites infecting sheep was (41.78%) , which is consistent with the findings of Peter *et al.* [4] in Kenya and Toulah [30] in Saudia Arabia. However, these results were lower than those obtained in Egypt by Mohamaden *et al.* [18] in Suez, (57.7%), Hassanen *et al.* [10] in Sharkia, (60.9%), Mohamed *et al.* [2] in Minya, (51.43%), and El-Alfy *et al.* [1] in Dakahlia, (68.4%). This may be due to factors such as animal health, management (feeding and housing), the use of anti-coccidial drugs, and other factors like illness and stress, experience, and the skills of the laboratory technician.

Concerning the prevalence of *Eimeria* species among sheep in relation to age, the current study revealed that the prevalence of *Eimeria* species among sheep was higher in younger animals compared to adults. Specifically, animals under one year of age had the highest infection rate at 72.7%, followed by those aged one to two years at 48%, and those over two years at 20.6%. This finding aligns with the results obtained by Mohamed *et al.* [2] in Minya Governorate, Egypt, where the highest infection rate was found in young sheep aged less than one year (73.6%), followed by yearlings (60.34%), and the lowest rate in adults (31.74%). Additionally, other studies conducted in Egypt [10, 18, 31], in Iran [16], in Nigeria [5], in Iraq [32, 33] also confirm that younger lambs are more susceptible to the disease than adults. As explained, lambs lack sufficient immunity to defend initial infections. However, older lambs can develop immunity and resist reinfection [8, 10, 14, 15]. However, this finding contrasts with the results reported by Barre *et al.* [34] in Somalia, where animals over one year of age had a higher prevalence rate (62.5%) than those under one year of age (20.8%). Moreover, Albayati *et al.* [35] in Iraq found that adults had a higher infection rate (60.9%) compared to lambs (53.6%).

The current study examined the prevalence of *Eimeria* species among sheep based on sex and revealed that

females exhibited a higher infection rate (44%) than males (33.3%). This finding aligns with earlier research conducted by Barre *et al.* [34], which revealed that male infection rates (41.7%) were significantly lower than female infection rates (58.3%). Other studies, such as Etsay *et al.* [20] found higher female prevalence compared to male prevalence (56.50%, 44.6%, respectively), Also, Hassanen *et al.* [10] demonstrated that male prevalence was (52.2%) lower than female prevalence (65.9%). Elkhtam *et al.* [31] also reported higher female prevalence than male prevalence (25.4%). This disparity may be attributed to the fact that females are more prone to infections due to the physiological stress experienced during pregnancy, lambing, and lactation [10]. However, this finding contradicts the results of studies conducted in Iraq by Hasan *et al.* [32], who found that there were significant differences between male and female hosts (65.42% vs. 45.69%), and Albayati *et al.* [35], who found that *Eimeria* species were more common in male hosts than females (62% vs. 44.4%) in lambs, but in adult, female sheep were more infected than males (77.9% vs. 41.3%). In contrast, Minnat [33] and Adeyemi *et al.* [36] indicated there was no statistically significant variation in infection rates across the sexes.

In terms of the season, the present study revealed that winter (70%) had a higher infection rate than fall (41.9%), spring (39.3%), and summer (24.4%). This finding was supported by Mohamed *et al.* [2], who reported that the cold season had a higher prevalence (74.24%) than the hot season (35.61%). The highest infection rate in winter has been attributed to several environmental factors, such as temperature, humidity, and rain, all of which affect the sporulation time of *Eimeria* oocysts. [10, 15]. However, this result differed from the findings of Elkhtam *et al.* [31], who reported that the maximum frequency was observed in fall (51.5%), with the lowest prevalence occurring in winter (16%), spring (28.5%) and summer (31.3%).

Conclusion

The present study offers significant findings regarding the occurrence and morphology of *Eimeria* species in sheep while simultaneously examining the risk factors of age, sex, and season. Our results indicate that age and season significantly influence the prevalence of *Eimeria* species in sheep in Sharkia Governorate. Furthermore, this study emphasizes the need for additional research to develop effective preventive and control strategies. Future investigations should concentrate on elucidating the molecular mechanisms responsible for the pathogenesis of *Eimeria* species in sheep and exploring potential vaccine candidates to prevent infection.

Conflict of interest

Authors declares no conflict of interest.

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الملخص العربي

معدل انتشار الكوكسيديا في الاغنام فى محافظة الشرقية- مصر

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أجريت هذه الدراسة لتحديد مدى انتشار طفيليات الكوكسيديا في الأغنام بمحافظة الشرقية - مصر. تم تجميع عدد (146) عينة براز من المناطق الريفيه في مركز بلبيس ومركز منيا القمح في الفترة من مايو 2023 حتى أبريل 2024. وسجلت الدراسة الحالية نسبة انتشار إجمالية لكوكسيديا الاغنام بلغت (41.7%). وان الحيوانات التي يقل عمرها عن سنة لديها نسبة إصابة عالية (72.7%) تليها الحيوانات من سنة إلى سنتين (48%) ثم الحيوانات أكثر من سنتين (20.6%) وأعلى نسبة إصابة بطفيل الكوكسيديا كانت في فصل الشتاء (70%) وأقل إصابة في فصل الصيف (24.4%) وكانت نسبة الإصابة بين الإناث أعلى (44%) مقارنة بالذكور (33.3%). بناءا على الخصائص المورفولوجية للبويضات المبوغة (sporulated oocyst)، تم تسجيل عشرة أنواع E. ahsata، E. bakuensis، E. faurei، E. granulosa، E. ovinoidalis، E. parva، E. crandallis، E. weybridgensis، E. marsica, E. pallida وكان النوع الأكثر انتشاراً هو E. bakuensis حيث بلغت نسبته (47.5%). كما يتضح من النتائج أن عمروجنس الحيوان بالاضافه الي الموسم هي أهم العوامل المتعلقة بإمكانية ودرجه الإصابة بالكوكسيديا. وأن ما يقرب من نصف الأغنام في الشرقية مصابة بمرض الكوكسيديا ولذلك فإنه لابد من وجود استراتيجية للسيطرة على المرض في مصر.