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Morphological Characterization of *Eimeria* spp. Infecting Imported Sawakni Sheep Linnaeus, 1758 (*Ovis aries*) in Riyadh City, Saudi Arabia

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ABSTRACT

Background: The epidemiology of parasitic infestations in sheep breeds is an important topic in meat production and human health. Protozoan parasites from the genus *Eimeria* cause coccidiosis. Coccidiosis ranks among the most perilous illnesses affecting sheep and other animals causing economic losses globally.

Methods: The study was conducted to identify and study species of *Eimeria* in *Ovis aries*, in Riyadh City, Kingdom of Saudi Arabia, from 1/1/2024 to 30/5/2024. A total of 1000 fecal samples of *Ovis aries* with different ages and genders were collected from Sheep markets and slaughterhouses in Riyadh city and employed morphological characteristics to accurately identify *Eimeria* spp.

Result: The results showed 5 species of *Eimeria* that infect sheep: *E. faurei* (length 30±0.5 µm; width 22±0.12 µm), *E. intricata* (length 45±0.83 µm; width 34±0.37 µm), *E. parva* (length 17±0.56 µm; width 16±0.75 µm), *E. granulosa* (length 30±0.18 µm; width 20±0.31 µm) and *E. pallida* (length 17±0.50 µm; width 13±0.50 µm). Moreover, the results showed that the infection rate was 35.6%. Summer recorded the highest percentage at 46.8%, while winter recorded the lowest at 26.4%. The species, *E. pallida* recorded the highest infection rate of 7.70%, while the species *E. intricata* represented the lowest infection rate of 0.30%. The current study provides relevant information to assess potential infections and future defenses against sheep coccidiosis, thereby minimizing financial losses for Saudi Arabia's sheep industry.

Key words: *Eimeria* Spp., Morphology, Oocysts, Sawakni sheep, Sporozoites.

INTRODUCTION

Livestock continues to dominate the Saudi economy and imports. Approximately 80% of the rural population living in remote villages is involved in animal husbandry. Sheep and goat meat constitute a major component of the daily diet of the citizens of Saudi Arabia (Abdel-Baki and Al-Quraishy, 2013; Akhtar *et al.,* 2023). The local production of meat is not sufficient for the needs of the Saudi market, so the officials facilitated the process of importing some types of sheep, such as the Al-Sawakni sheep, to cover the deficit in the domestic product (Al-Owaimer *et al.,* 2017). Al-Sawakni sheep are spread in southern and southeastern Africa, including Sudan and Ethiopia. Sudan is considered one of the largest producers of livestock in Africa and the Arab world. In 2007 AD, estimates placed the number of sheep in Sudan at about 51 million heads, with an estimated annual growth rate of 3.10%. Sheep breeding is considered the main economic source in Sudan (Elrasheed *et al.,* 2010). Gastrointestinal (GI) parasitism is considered one of the most serious and underestimated problems that hinder sheep, cattle and swamp buffalo productivity (Perry and Randolph, 1999; Das *et al.,* 2018). Moreover, studies have demonstrated the importance of public health of some sheep GI parasites, implicating them in zoonotic transmission to humans through direct contact with sheep manure or indirect ingestion of contaminated manure (Perry and Randolph, 1999). Small ruminants can get coccidiosis, which is caused by coccidia parasites from the genus *Eimeria*. These parasites live in the small and large intestines and mostly affect young animals (Maratea and Miller, 2007). Several species of *Eimeria* are involved in different ruminant hosts (bovine, ovine and caprine) but there is no cross-infection due to the strict host specificity. Coccidiosis is of great economic importance because of the losses due to clinical disease (diarrhea), subclinical infections and poor weight (Maratea and Miller, 2007; Murshed *et al.,* 2023). Coccidiosis is one of the most important diseases that may affect sheep (Koura *et al.,* 2001). Infection with this parasite causes severe diarrhea and a tear in the intestinal wall of infected sheep, which

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reduces their efficiency in digesting and absorbing food and, in turn, disrupts the natural growth process and emaciation, reducing their productivity and economic value (Mehlhorn, 2001; Kaur *et al.,* 2019). In view of the pathogenic importance of *Eimeria* parasites, the Kingdom of Saudi Arabia conducted survey studies on *Eimeria*, a parasite that infects local sheep in general, without specifying the specific breeds (Kasim and Al-Shawa, 1985; Toulah, 2007). Large-scale imports of the Al-Sawakni breed of sheep from various regions into the Kingdom of Saudi Arabia are known to occur, which is potentially introducing new parasites into the Saudi environment. It is worth noting that researchers studied other types of coccidiosis of the genus Sarcocystis that infect some animals slaughtered in the city of Riyadh in the Kingdom of Saudi Arabia and proved that Al-Sawakni sheep were infected with this type of parasite (Al-Quraishi, 2005). Therefore, this study focused on surveying and determining the rates of infection with coccidia parasites of the genus *Eimeria* in the Al-Sawakni sheep breed located in the city of Riyadh, as well as studying the internal stages of these parasites whenever possible to understand the parasite's effect on the host and the results of the parasitism process. This study aimed to identify coccidia parasites of the genus *Eimeria* that infect Sawakni sheep in the city of Riyadh.

MATERIALS AND METHODS

The sample collection area

A total of 1000 samples of manure from Sawakni sheep were collected at a rate of 50 samples every week for 20 weeks from 1/1/2024 to 30/5/2024. The fecal samples were handled according to the method described by Upton *et al.* (1991), each sample was placed in a tube and the necessary data was written on it. It was then transferred to the parasite laboratory at the Department of Zoology, College of Science, King Saud University for further investigations.

Study animals

The samples of dung from Sauakni sheep (*Ovis aries*) were collected from livestock markets, barns, grazing areas and all slaughterhouses in the city of Riyadh, Kingdom of Saudi Arabia. Sawakni is regarded as a sheep with a long, thin tail, full of fat and a clear pulp below the neck (Fig 1). These sheep are fast-growing as well as fast multiplying and mostly can produce twins at an early age (Snyman, 2014).



Fig 1: A photograph of sawakni sheep (*Ovis aries*).

Detection of natural coccidian infection

Each sample was microscopically examined separately to confirm its infection or non-infection by preparing a temporary dung swab. Thereafter the samples were classified based on their infection or non-infection with the primary vesicles of coccidia parasites. Collected fresh oocysts from the infected sheep's feces were concentrated using the floatation technique, *i.e.* putting 2 g of each sample into 50 ml of saturated saline. The suspension was passed through cheesecloth and centrifuged at a speed of 1500 rpm for 10 minutes (Long *et al.,* 1976). To find out how long it takes for oocysts to sporulate and what shape they take, freshly collected oocysts that hadn't already sporulated were mixed with 2.5% (W/V) aqueous potassium dichromate, in Petri dishes and left to sporulate at room temperature (25±2°C). The samples were examined periodically under the microscope using an oil immersion objective lens and recorded the progress of sporulation.

Identification of *Eimeria* species and examination of microscopic

Used an Olympus microscope (model number Olympus CX41, made in Tokyo, Japan) with a digital camera and an oil immersion lens to look at the shape and properties of sporulated oocysts. The length and width of the oocyst, the size of the sporocysts, the presence or absence of residual bodies of sporocysts and the presence or absence of Stieda bodies were also used to identify the *Eimeria* species of at least fifty sporulated oocysts. In addition, the presence or absence of Stieda bodies was also employed. Each and every measurement was carried out using an ocular micrometer in conjunction with an oil immersion lens. The measurements were given in micrometers as means ±SD followed by the range in parentheses. Only oocysts with sharply defined outlines and intact sporocysts were measured and recorded.

Statistical analysis

The mean as the mean ± standard error of the mean (SEM) was used to express the values. The statistical analysis was performed using a one-way analysis of variance (ANOVA). A p-value ≤0.05 was considered statistically significant.

RESULTS AND DISCUSSION

In this study, 1000 samples of Sawakni sheep (*Ovis aries*), were examined during the period 1/1/2024 to 30/5/2024, from the livestock markets and various slaughterhouses in the city of Riyadh. Sheep are considered one of the most important components of livestock and a major source of meat in the Arabian Peninsula in general and in the Kingdom of Saudi Arabia in particular. However, they are

infected with diseases that reduce their productivity. Coccidiosis is one of the most important diseases that may affect sheep (Dudko *et al.,* 2018). Infection with this parasite leads to severe diarrhea and a tear in the intestinal wall of infected sheep, which reduces their efficiency in digesting and absorbing food and, in turn, leads to disruption of the natural growth process and emaciation, which reduces the productivity and economic value of these sheep (Akhtar *et al.,* 2023). In view of the pathogenic importance of *Eimeria* parasites, some survey studies were conducted in the Kingdom of Saudi Arabia on *Eimeria*, which infects types of local sheep in general, without specifying the breeds of these sheep (Kasim and Al-Shawa, 1985; Toulah, 2007). Earlier to this (Omer *et al.,* 2020) reported that sheep in the city of Riyadh in the Kingdom of Saudi Arabia were infected with coccidian.

After examining all the study samples, infected with coccidia parasites of the genus *Eimeria* the average total infection rate was 35.6% (Fig 2), the highest infection rate was in summer at 46.8% and the lowest infection rate was 26.4% in winter (Table 1).

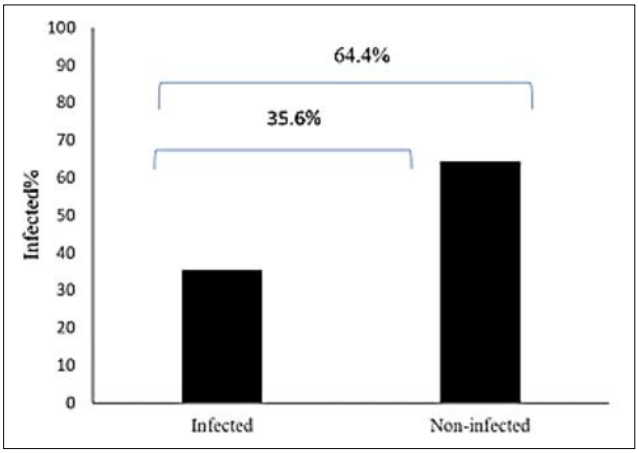


Fig 2: The infection rate of *Eimeria* parasites in Al-sawakni sheep.

Table 1: Infection rates with the *Eimeria* parasite in Al-sawakni sheep according to year-seasons.

Season	Samples examined	Infected samples	Infected%
Autumn	250	91	36.4%
Winter	250	66	26.4%
summer	250	117	46.8%
Spring	250	82	32.8%
Total	1000	356	35.6%

Table 2: The number of infections with *Eimeria* types and the percentage of infection.

Eimeria spp.	No. infected samples	Infected %
Eimeria pallida	77	7.70%
Eimeria faurei	76	7.60%
Eimeria granulosa	53	5.30%
Eimeria parva	52	5.20%
Eimeria intricata	3	0.30%

In all five different species have been identified: *E. pallida, E. faurei, E. granulosa, E. parva* and *E. intricata* where the infection was always mixed in all infected samples, the infection with *E. pallida* was recorded by 7.70%, which was the highest percentage of the species in the infected samples, while the infection with *E. intricata* by 0.30% was the lowest percentage of infection with this type (Table 2).

Based on the morphometric data, five *Eimeria* species were detected in the feces of the examined sheep and identified as follows:

As for the first type *E. faurei*, the sporulated oocysts were oval in shape and had a yellow-brown wall. These vesicles ranged in length from 30±0.5 (31-29) μm, while the width of the ovoid oocysts was 22±0.12 (21-23) μm and the ratio of length to width was (shape index) 1.40 (1.3-1.5) μm.

While examining the sporulated oocytes, it was found that there was no micropyle hole, micropyle cap, oocyst residuum, but there was presence of a polar granule. It was also found that each vesicle contains four 4-sporocysts and 2-sporozoites and has an ovoid shape, measuring 15±0.62 (15-17) μm in length and 7±0.81 (7-8) μm in width and the aspect ratio (shape index) was 2 (2-2.15) μm. The study proved that the sporocysts do not contain a steida body and contain the sporocyst residuum, as shown in (Fig 3A). The parasite *Eimeria faurei* was described for the first time in sheep and goats (Moussu and Marotol, 1902) and when comparing the results of this study with the results of the researchers (Hasan and Abed, 2012), as shown in Table 3. There were no significant differences when compared with the results of Moussu and Marotol (1902) that also showed the concordance of the description for the parasite *E. faurei*.

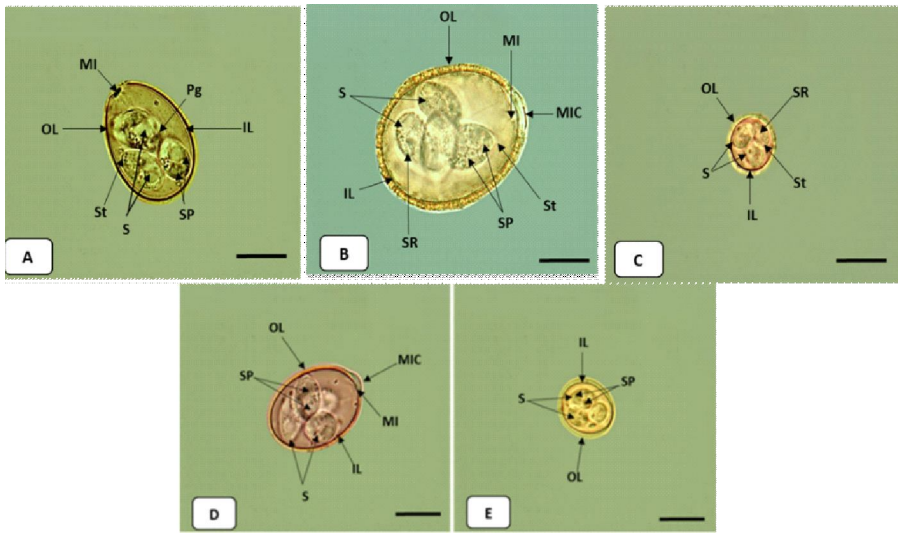
As for the second type *E. intricata* (Fig 3B), the sporulated oocysts are characterized as one of the largest types of *Eimeria* that infect sheep. They are ellipsoidal in shape and have a rough brown wall that is striped. The measurements of these oocysts range in length from 45±0.83 (43-48) μm, while the width of the oocysts is 34±0.37 (34-35) μm and the aspect ratio (shape index) is 1.3 (1.3-1.4) μm. Upon examining the sporulated oocytes, the presence of a micropyle, the presence of a micropyle cap and the absence of an oocyst residuum were noted. It was also found that each oocyte contains four sporocysts, which are diploid (2-sporozoites) and have an ovoid shape and their measurements range from 13±0.93 (13-16) μm in length and 8±0.75 (8-10) μm in width and the aspect ratio (shape index) was 1.6 (1.5-1.6) μm. The study showed that the sporocysts contain the steida body as well as the sporocyst residuum (Fig 3B). The parasite *Eimeria intricata* was described for the first time in sheep (Spiegl, 1925). The present study in regard to *E. intricata* was in agreement with Toulah (2007), while there are some slight differences

in the sizes of sporocysts in the current study compared with a study of Hasan and Abed, (2012).

The third type is *Eimeria parva* (Fig 3C), the sporulated oocysts are spherical in shape with smooth bilayers, ranging in color from pale yellow to greenish yellow. The length of these oocytes ranged from 17±0.56 (13-19) μm, while the width of the oocytes was 16±0.75 (12-18) μm and the length-to-width ratio was 1 (1-1.1) μm. When examined the sporulated oocytes, it was found that there was no micropyle, no micropyle cap and no remnants of oocyst residuum. It was also found that each oocyte contains four sporocysts that are diploid (2-sporozoites) and have an ovoid shape, measuring 8±0.75 (8-10) μm in length and 6±0.1 (5-6) μm in width and the proportion of the length to width (shape index) was 1.4 (1.4-1.6) μm. The study showed that sporocysts contain a small steida body, as well as sporocyst residuum as indicated in Fig 3C. It was described for the first time in sheep by (Kotlan *et al.,* 1929). In the present study, it was confirmed that Al-Sawakni sheep in Saudi Arabia were infected with this parasite which matches with the description of the parasite *E. parva* in many studies (Dittmar *et al.*, 2010; Hasan and Abed, 2012).

As for the fourth type *Eimeria granulosa* (Fig 3D), the sporulated oocysts are ovoid and have a yellow, transparent wall. The length of these oocysts ranged from 30±0.18 (29-31) μm, while the width of the oocysts was 20±0.31 (19-21) μm and the ratio of length to width (shape index) was 1.4 (1.4-1.5) μm. When examining the sporulated oocysts, it was revealed that there was a micropyle, the presence of a micropyle cap and the absence of an oocyst residuum. It was also found that each oocyst contains four sporocysts that are diploid (2-sporozoites) and have an ovoid shape, measuring 11±0.93 (11-12) μm in length and 6±0.93 (6-7) μm in diameter. The width, aspect ratio (shape index) was 1.7(1.7-1.75) μm. The study showed that sporocysts contain the steida body as well as the sporocyst's residuum. The parasite *Eimeria granulosa* was described for the first time by Christensen (1938) in sheep and in this study, it was proven that Al-Sawakni sheep in Riyadh, Saudi Arabia, were infected with this *E. granulosa*. Some slight differences in oocyst sizes were found between the present study (Table 3) and reports by Dittmar *et al.* (2010) and Hassan and Abed, (2012).

The fifth species is *Eimeria pallida* (Fig 3E), where, the sporulated oocysts are oval and have a yellow-green wall. The length of these oocysts ranges from 17±0.5 (14-19) μm, while the oocytes are 13±0.50 (11-14) μm wide and the aspect ratio is 1.3.(1.2-1.4) μm. When examining the sporulated oocysts, it was found that there was no micropyle, no micropyle cap and no oocyst residuum. It was also found that each oocyst contains four sporocysts that are diploid (2-sporozoites) and have an oval shape, measuring 6±0.75 (6-8) μm in length and 4±0.1 (3-6) μm in width and the aspect ratio (shape index) was 1.6 (1.6-1.75) μm. The study proved that the sporocysts contain the solid body as well as the remaining sporocysts. The parasite *Eimeria pallida*, was described in sheep for the first time by Christensen, (1938) which is in agreement with the present study where this parasite was seen in Al-Sawakni sheep, which again agrees with the study of Dittmar *et al.*, (2010); Hasan and Abed (2012). The current study recorded a total infection rate with *Eimeria* parasites that reached 35.6% in the Sawakni sheep in the city of Riyadh, where the highest infection rate was in summer at 46.8% and the



E. faurei. (Fig 3A): *E. intrícata;* (Fig 3B): *E. parva;* (Fig 3C): *E. granulosa;* (Fig 3D): and E. *pallida* (Fig 3E). Where, Inner layer is denoted by (IL), Micropyle by (MI), Micropyle cap by (MIC), Outer layer by (OL), Polar granule by (Pg), Sporocyst by (S), Sporozoite by (SP), Sporocyst residuum by (SR) and Steida body by (ST). Scale bars=10 μm.

Fig 3: Sporulated oocysts of the five species of *Eimeria* collected from naturally infecting Sawakni Sheep (*Ovis aries*).

Table 3: Comparison between the *Eimeria* parasites obtained through the current study and previously recorded studies in sheep.

Species	Oocyst				Sporocyst			Location	Source
	Measurements	M	MC	OR	Measurements	SB	SR		
	28-36 × 21-27.5	+	-	-	12-14 × 8-9.5	+	-	USA	Moussu and Marotol, 1902
Eimeria faurei	28-31 × 21.3-32.5	+	-	no	13.1-17.5 × 9.3-12.5	no	No	Iraq	Hasan and Abed, 2012
	30.5-31 × 22.12-23	+	-	-	9.75-13 × 8.37-11	+	-	Saudi	This study
	46-53 × 31-34	+	+	no	16-18 × 8-10	+	+	Saudi	Toulah, 2007
Eimeria intricata	42.30-50 × 29.9-35	+	+	no	17.4-20 × 11.6-17.5	no	No	Iraq	Hasan and Abed, 2012
	45.83-48 × 34.37-35	+	+	-	13.93-16 × 8.75-10	+	+	Saudi	This study
	16.79-20 × 15.55-19	no	no	no	7.54-11 × 5.08-	no	No	Germany	Dittmar *et al.*, 2010
Eimeria parva	18.5 × 16.8-17	-	-	no	7.50-12.5 × 6.2-7.5	no	No	Iraq	Hasan and Abed, 2012
	17.56-19 × 16.75-18	-	-	-	8.75-10 × 5-6	+	-	Saudi	This study
	32.3-40 × 22.9-27	no	no	no	14-18 × 8.21-10	no	No	Germany	Dittmar *et al.*, 2010
Eimeria granulosa	25.8-30 × 20-22.5	+	+	no	11.2-15 × 8-10	no	No	Iraq	Hasan and Abed, 2012
	30.18-31 × 20.31-21	+	+	-	11.93-12 × 6.93-7	+	+	Saudi	This study
	15.86-20 × 12.36-14	no	no	no	7.19-9 × 4.64-6	no	No	Germany	Dittmar *et al.*, 2010
Eimeria pallida	16.5-17.5 × 11.3-15	-	-	no	7.30-10 × 5.2-7.5	no	No	Iraq	Hasan and Abed, 2012
	17.5-19 × 1.5-14	-	-	-	6.75-8 × 4.1-6	+	+	Saudi	This study

M = Micropyle; MC = Micropyle cap; SR = Oocyst residuum; SB = Stieda body; SR = Sporocyst residuum. + = Present; - = Absent; no= No data.

lowest infection rate in winter was 26.4%, the infection was mixed with more than one type of *Eimeria* in all infected samples. It was noted in this study that there is a decrease in the infection rate in the winter season and the temperature may reach below zero degrees Celsius at times, in contrast to the summer, which makes the sporulation process impossible on the ground (Parker and Jones, 1990).

CONCLUSION

The present survey shows the prevalence of coccidiosis in sheep in Riyadh City, Saudi Arabia. Knowledge of the current prevalence of coccidiosis and *Eimeria* species will help for assess the potential for infection and provide relevant data to evaluate the effectiveness of future control strategies for coccidiosis in sheep, thereby minimizing economic losses in sheep breeding.

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Conflict of interest

The authors declare no conflict of interest.

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