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MICROSCOPIC DETECTION OF COCCIDIOSIS IN SMALL RUMINANTS IN WASIT PROVINCE, IRAQ

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Abstract

Background and aim: Coccidiosis is a host-specific protozoan disease caused by several species of Eimeria genus, resulting in variable economic drawbacks due to loss in performance and even mortality. The current study investigated the prevalence rate of coccidiosis in goats and sheep in Wasit province (Iraq), morphological detection of the main Eimeria species found in these animals, and identification of the relation between positive infections and specific epidemiological risk factors. Materials and methods: Fresh fecal samples of 300 animals involving 150 goats and 150 sheep of various ages and sexes were collected during January-December (2022), prepared, and examined microscopically. Results: The overall prevalence rate of coccidiosis among study animals was 32.33%, including 38.67% in goats and 26% in sheep. Based on their morphology, 7 and 4 Eimeria species were identified in goats and sheep, respectively, with a significant prevalence of E. arloingi in goats and E. ahsata in sheep. Mixed infection patterns appeared significantly in goats and sheep compered to single and bilateral patterns. An insignificant association between body temperature, pulse, and respiratory rates of infected and non-infected animals was observed, while values of body condition score were recorded as poor scores in goats and medium scores in sheep. Age, sex, region, and season showed a significant association with coccidiosis. Conclusion: Coccidiosis remains highly prevalent, subclinical

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parasitic disease in small ruminants in particular goats. Therefore, successful and economical control can be achieved once detailed knowledge about management characteristics and the time course of infection in a given flock is collected. Also, implementing of advanced diagnostic assays such as molecular phylogeny could help increase the sensitivity and specificity of an assay in identifying different Eimeria species and their association with other local and global strains.

Keywords: *Eimeria, Coccidian diseases, Morphological diagnosis, Goat, Sheep*.

Introduction

Coccidiosis is a host-specific protozoan disease caused by many species of the *Eimeria* genus belonging to the *Eucoccidiorida* order under the *Apicomplexa* phylum (Gondipon and Malaka, 2021). Worldwide, the disease represents one of the most important epidemic diseases in different animals and is distributed in many countries, especially in high-density flocks and in those having a high chance of direct contact with their feces (Bangoura et al., 2022; Lianou et al., 2022). Many researchers have confirmed that the parasite is obligatory and needs one host to complete its life cycle and begins ingesting the contaminated foods or water with sporulated oocysts (Lindsay et al., 2019; Di Genova and Knoll, 2021; Mohammed et al., 2021). The oocysts ruptured to sporozoites that invaded the intestinal epithelial cells and transferred into trophozoites ،then to primary schizonts containing the primary merozoites. After invading the intestinal epithelium, secondary schizonts containing secondary merozoites were produced by asexual reproduction, and then, the second generation of merozoites produced the macro- and micro-gamonts by sexual reproduction (Majeed et al.,2020; Mohammed et al., 2021).

Coccidiosis is economically important in many animal farms, especially small animals, because of the clinical effects of parasites that cause diarrhea, dehydration, obesity, anemia, and medical costs. Also, it causes a loss in performance and even mortality, especially when it is complicated by bacterial infections (Dash et al., 2020; Mohamed et al., 2022). Recently, 16 and 15 coccidian species were identified in goats and sheep, respectively, and varied largely according to the time of formation and growth of oocytes, site of infection as well as the oocyte maturation (de Macedo et al., 2019; Mohamed et al., 2023). For diagnosis, various traditional methods were applied to determine the parasite or its species based on intensive macroscopic observations of fecal samples, clinical signs of infected animals, and morphological aspects of eggs detected under the microscope. In addition, serological and molecular techniques have been provided as active alternative tools for identifying and confirming of coccidian species, but at excessive cost (Lindsay and Dubey, 2020; Khairelsiad et al., 2021).

In Iraq, several studies have been done in sheep and/or goats with variable prevalence rates and different *Eimeria* species in Al-Muthana (Mohammed, 2013), Babylon (Rabee et al., 2020), Baghdad (Qasim and Al-Zubaidi, 2022), Basrah (Shaheed and Al-Azizz, 2020),

Diyala (Minnat, 2014), Duhok (Al-Bayati et al., 2016), Mosul (Hasan and Abed, 2012), Salah Al-Din (Aziz and Mahmoud, 2020), Sulaimaniya (Kareem and Yücel, 2015), and Wasit (Al-Saadoon and Al-Rubaie, 2018 a; b). The current study was carried out to investigate the prevalence rate of coccidiosis in goats and sheep in Wasit province (Iraq), morphological detection of the main *Eimeria* species found in these animals, and identification of some related between positive infections and some epidemiological risk factors.

Materials and methods

Samples

A total of 300 animals, 150 goats and 150 sheep, of different ages and sexes were selected randomly from different areas in Wasit province (Iraq) during January-December (2022). Fresh feces samples were taken from all animals subject to the study; approximately 10-30 grams were transferred into labeled plastic containers, transported under cooled conditions using a plastic ice box, and kept at 4°C until examined.

Clinical examination

Clinical data were recorded, including body temperature, heart and respiratory rates, and diarrhea. Body condition score was estimated in goats (Ghosh et al., 2019) and sheep (Kenyon et al., 2014). Epidemiological data, including age, sex and body score conditions of animals, region, and season of samples collection, were recorded. Based on Al-Ansari (2021), the season factor was categorized in this study into two periods: dry (April to September) and wet (October to May).

Microscopic examination of fecal samples

Direct drop on sslide preparation and floatation in saturated salt solution (sodium chloride) using the modification of McMaster, in addition to sporulation of oocysts in 2.5% and 5% potassium dichromate at room temperature (26-33°C) were applied to diagnosis and classification of different *Eimeria* species at ×10 and ×40 of light microscope (MEIJI, Japan), (Chartier and Paraud, 2012; Al-Bayati et al., 2016).

Statistical analysis

The t-test and One-Way ANOVA were used for statistical analysis of all collected data using the GrpahPad Prism Software version 6.0.1 (GrpahPad Software, Inc, USA). Values were represented as Number (No.) and Percentage (%) or as Mean (M), Standard Error (SE) and Range (R). The variation in the results between the studied groups was considered significant at $P<0.05$ value (Gharban, 2023).

Results

Microscopic data

The overall prevalence rate of *Eimeria* spp. among study animals tested microscopically was 32.33% (97 of 300), involving 38.67% (58 of 150) in goats and 26% (39 of 150) in sheep (Table 1).

Table (1): Microscopic examination of *Eimeria* species infection among study animals

Animal	Total No.	Value [No. (%)]	
		Positive	Negative
Goat	150	58 (38.67%)	92 (61.33%)
Sheep	150	39 (26%)	111 (74%)
Total	300	97 (32.33%)	203 (67.67%)
p-value		***0.0373***	-

Based on their morphology, *E. arloingi* (39.73%) was increased significantly (P<0.0181) in study goats, while *E. caprovina* (2.74%) and *E. aspheronica* (5.48%) were decreased significantly in comparison with other species; *E. alijevi* (16.44%), *E. chirstenseni* (15.07%), *E. jolchijevi* (12.33%), and *E. ninakohlyakimovae* (8.22%). In sheep, *E. ahsata* (38.18%) was prevalent significantly (P<0.0358) among other detected *Eimeria* species; *E. parva* (25.46%), *E. ovinoidalis* (16.36%) and *E. granulosa* (20%), (Figure 1).

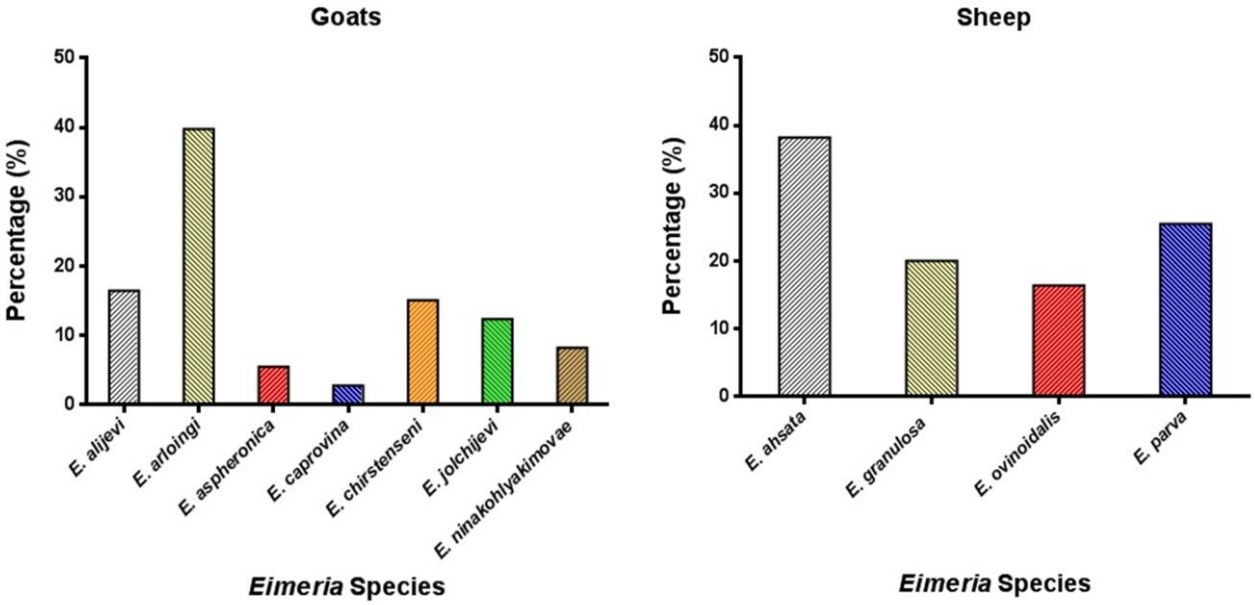

Figure (1): Morphological detection of *Eimeria* species infection in positive study animals

Concerning pattern of infection in infected animals, mixed infections were significantly (0.0099 and 0.0089, respectively) higher in both goats [77.59% (45/58)] and sheep [69.23% (27/39)] than bilateral [18.96% (11/58) and 20.51% (8/39), respectively] and single [3.45% (2/58) and 10.26% (4/39), respectively] infections (Figure 2).

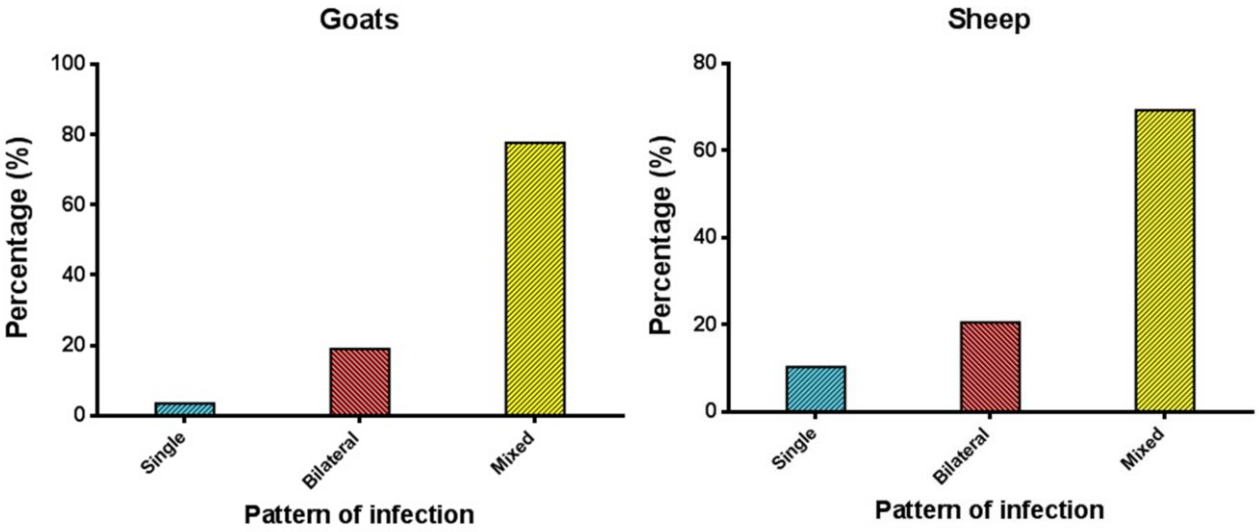

Figure (2): Pattern of infection by different *Eimeria* species in positive goats and sheep

Clinical examination

Although no significant alteration in body temperature, pulse, and respiratory rates was shown between infected and non-infected animals (Tables 2, 3), values of body condition score exhibited a higher prevalence rate of bad score in goats (36.21%) and medium score in sheep (38.46) than other scores. However, excellent score was reduced significantly in both goats (1.72%) and sheep (7.69%) as in (Table 4).

Table (2): Clinical results of vital signs in goats (Total No: 150)

Symptom	**Value [M ± SE (R)]**		***p-value***
	Positive	**Negative**	
Body temperature / °C	39.11 ± 0.16 38.27 – 40.69	39.52 ± 0.13 38.48 – 41.02	*0.0976*
Heart rate / Minute	81.61 ± 3.42 69 - 89	79.85 ± 2.97 67 - 91	*0.093*
Respiratory rate / Minute	25.5 ± 0.93 22 - 37	22.25 ± 0.71 23 - 35	*0.0659*

Table (3): Clinical results of vital signs in sheep (Total No: 150)

Symptom	Value [M ± SE (R)]		p-value
	Positive	Negative	
Body temperature / °C	38.34 ± 0.21 38.52 – 41.04	38.65 ± 0.25 38.26 - 39.97	0.074
Heart rate / Minute	78. 25 ± 1.65 59-88	77.76 ± 2.01 61 - 90	0.08
Respiratory rate / Minute	23.16 ± 1.23 19 - 31	21.93 ± 1.37 18 - 32	0.0828

Table (4): Clinical association of body condition score and diarrhea to *Eimeria* species infection in the goats (58) and sheep (39)

Symptom	Value [No. (%)]			
	Goats		Sheep	
Body condition score	**No.**	**%**	**No.**	**%**
1 (Bad)	21	36.21	9	23.08
2 (Medium)	17	29.31	15	38.46
3 (Good)	14	24.14	8	20.51
4 (Very good)	5	8.62	4	10.26
5 (Excellent)	1	1.72	3	7.69
p-value		***0.042***		***0.0254***
Existed Diarrhea	13	22.41	7	17.95
Absent Diarrhea	45	77.59	32	82.05
p-value		***0.0368***	-	***0.0331***

Risk factors

Four risk factors were tested for their association with coccidiosis positivity: age, sex, region, and season (Tables 5-8). Among different age categories, the significantly highest results were detected in goats and sheep aged ≤ 6 months (64.58% and 43.08%, respectively) and decreased significantly with advancing age (P<0.0109 and P<0.0176). Concerning sex, female goats and sheep were showed significant increase in positivity (42.2% and 28.87%, respectively) than males (29.27% and 20.75%, respectively), (P<0.0397 and P<0.0403, respectively).

Significantly, goats and sheep reared in rural areas were revealed a higher positive values (43.31% and 33.96%, respectively) than those in sub-urban (17.65% and 6.67%, respectively) and urban (0% and 7.14%, respectively) regions (P<0.0146 and P<0.0271,

respectively). Significantly lowered rate of coccidiosis in goats and sheep was seen in the dry season (32.35% and 20.37%, respectively) compared to the wet season (43.9% and 29.17%, respectively), (P<0.0392 and P<0.0413, respectively).

Table (5): The relationship between the age and coccidiosis infection in goats and sheep

Age	Goats		Sheep	
	Total No.	Positive [No. (%)	Total No.	Positive [No. (%)
≤ 6 months	48	31 (64.58%)	65	28 (43.08%)
≥7-12 months	51	22 (43.14%)	43	9 (20.93%)
≥ 1-2 years	25	4 (16%)	29	2 (6.9%)
≥ 3 years	26	1 (3.85%)	13	0 (0%)
p-value	-	0.0109	-	0.0176

Table (6): The relationship between the sex and coccidiosis infection in goats and sheep

Sex	Goats		Sheep	
	Total No.	Positive [No. (%)	Total No.	Positive [No. (%)
Female	109	46 (42.2%)	97	28 (28.87%)
Male	41	12 (29.27%)	53	11 (20.75%)
p-value	-	0.0397	-	0.0403

Table (7): The relationship between the regions and coccidiosis infection in goats and sheep

Region	Goats		Sheep	
	Total No.	Positive [No. (%)	Total No.	Positive [No. (%)
Rural	127	55 (43.31%)	106	36 (33.96%)
Sub-Urban	17	3 (17.65%)	30	2 (6.67%)
Urban	6	0 (0%)	14	1 (7.14%)
p-value	-	0.0146		0.0271

Table (8): The relationship between the seasons and coccidiosis infection in goats and sheep

Season	Goats		Sheep	
	Total No.	Positive [No. (%)	Total No.	Positive [No. (%)
Dry	68	22 (32.35%)	54	11 (20.37%)
Wet	82	36 (43.9%)	96	28 (29.17%)
p-value	-	0.0392	-	0.0413

Discussion

Despite the progress in the methods of controlling and treating animals, goats, and sheep are still exposed to many parasitic infections that may cause serious health issues for the animals. Coccidiosis caused by *Eimeria* spp. is one of the most important parasitic infections, affecting these animals worldwide, including Iraq. The prevalence rate of coccidiosis reported in this study showed significant elevation of Eimeria infection in goats compared to sheep. In comparison with other local studies, there were 69.5% and 56.25% in goats and sheep, respectively in Tikrit City (Hasan and Mahmood, 2021), 67.5% in Al-Muthana (Mohammed, 2013), 39.16% in sheep in Babylon (Rabee et al., 2020), 85% in goats in Baghdad (Hasson and Al-Zubaidi, 2022), 38.9% in goats and 45.6% in sheep in Basrah (Shaheed and Al-Azizz, 2020), 87.34% in goats and 85.52% in sheep in Diyala (Minnat, 2014), 37.67% in goats in Duhok (Al-Bayati et al., 2016), 63.6% in sheep in Mosul (Hasan and Abed, 2012), 67.04 in sheep in Salah Al-Din (Aziz and Mahmoud, 2020), 72% in sheep in Sulaimaniya (Kareem and Yücel, 2015), and 57.5% in sheep in Wasit (Al-Saadoon and Al-Rubaie, 2018 a; b). There is a high prevalence of coccidiosis in sheep and/or goats that reported by all the previous studies which in line with the current study result showed the severe problem that this parasite poses and calls for its proper management. However, variations in the infection rate of the diseases might be attributed to different host, pathogen, and environmental factors.

Taxonomy genus of *Eimeria* could be classified according to oocysts sizes and morphological characteristics of oocysts and sporocysts in addition to the host from which these oocysts have been recovered. In this study, morphological diagnosis revealed that there were seven *Eimeria* species in goats and four in sheep, with a significant increase in the prevalence of *E. arloingi* and *E. ahsata* in goats and sheep, respectively. In other studies, there were four (Minnat, 2014), six (Al-Bayati et al., 2016), seven (Mohamaden et al., 2018), and 12 (Hasan and Mahmood, 2021) species in goats with a significant prevalence of *E. arloingi* as detected by other studies (Kheirandish et al., 2014; Mohamaden et al., 2018). Whereas in sheep, the number of *Eimeria* species reported by authors was eight (Minnat, 2014), 10 (Al-Rubaie and Al-Saadoon, 2018 a, b), 11 (Hasan and Mahmood, 2021), and 12 (Rabee et al., 2020) with a significant prevalence of *E. ahasta* in all of these studies.

Throughout this study, it was found that a mixed pattern of *Eimeria* species formed the highest rate of infection when compared to bilateral and single infections. These findings are consistent with many reports (Minnat, 2014; Al-Bayati et al., 2016; Mohamaden et al., 2018; Hasan and Mahmood, 2021). This might be because of the open grazing system and high contamination of pastures with different species of *Eimeria*.

There were no significant alteration in clinical signs of positively infected animals compared to negatives; however, the body condition scores of positive animals showed that the bad and medium scores were more prevalent in goats and sheep. Constable et al. (2016) mentioned that subclinical infections in goats and sheep are common, but no documented evidence shows that the growth rate is affected, even with high infection levels. Mohamaden et al. (2018) revealed no specific clinical signs of acute coccidiosis exhibited by infected animals, particularly in sheep that were seen as apparently healthy and alert with good appetite, but growth impairment and dullness were shown in some cases. Mohammed et al. (2021) reported that the appearance of clinical signs depends on the species of *Eimeria* and its location in the intestine, genetic susceptibility, and stress factors.

Age is considered a significant factor in coccidiosis as several studies found that the prevalence and severity of infection are high in the younger ages and decrease gradually with the advancing age of the animals. Numerous authors have reported heavy excretion of oocysts by kids and lambs aged between 2 and 4 months (Al-Bayati et al., 2016; Mohamaden et al., 2018), which might be attributed to the relative weakness of maternal immunity and stress during the weaning period at this time. In contrast, the low prevalence of coccidiosis with increasing age could be attributed to acquired immunity and/or resistance subsequent to infection exposure (Gharban et al., 2022).

Although few reports showed no relationship between *Eimeria* infection and the sex of the animal (Alemayehu et al., 2013; Etsay et al., 2020), other studies reported that female goats and sheep showed a higher receptivity to infection than males (Kareem and Yücel, 2015; Al-Rubaie and Al-Saadoon, 2018 a, b; Shaheed and Al-Azizz, 2020). The reason might be attributed to the fact that males in Iraq are usually used for slaughter; therefore, they receive more feeding attention and health care than females. Consequently, the number of males in all flocks is usually less than that of females, and this may affect in sampling method and, subsequently, its results. Also, females might have additional stresses as they exist in pasture for a longer period than males and hormonal changes due to milk reproduction, estrus, and gestation.

The influence of seasonal factors observed in this study could be relevant to hygienic aspects and resistance status through nutrition. Rainy season and cold stress have been observed to cause a heavier excretion of *Eimeria* oocysts, which leads to increasing the levels of infection as humidity is more favorable for sporulation of oocysts (Kumar et al., 2016; Mohamaden et al., 2018).

Conclusion

Coccidiosis remains a highly incidence, subclinical parasitic disease in small ruminants in particular goats. Age, sex, region, and season appear as important risk factors that might play a role in incidence of infection. Therefore, successful and economical control can be achieved once detailed knowledge about management characteristics and the time course of infection in a given flock is collected. Also, the implementation of advanced diagnostic assays as the molecular phylogeny could help increase the sensitivity and specificity of an assay in identify of different *Eimeria* species and its association with other local and global strains.

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