

Study of Subclinical Coccidiosis in Small Ruminants from Sulaymaniyah Province/ Iraq

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Abstract

Coccidiosis caused by *Eimeria* species, with great economic importance infecting numerous species including small ruminants. The current study aimed to determine the occurrence of *Eimeria* subclinical infection in Sulaymaniyah provinces, during March 2021 to February 2022. A total of 260 fecal samples (152 sheep and 108 goats) were collected from small ruminants' farms belong to four regions. Fecal floatation technique was applied for examination of fecal samples. Coprological examination revealed that *Eimeria* spp. parasitized the intestine of 133 animals with infection rate of 51.15%. Both sheep and goats represented subclinical coccidiosis with infection rates of 52.63% and 49.07% respectively. Mixed infection with more than one *Eimeria* spp. was dominant and found in sheep 60.00% and goats 40.00%. However, infection by single species was revealed in sheep 58.62% and goats 39.66% goats. The frequency of subclinical coccidiosis was higher among animals in Bakrajo 53.85%, and lower in Qaradagh 46.67%. The impacts of age on prevalence rate revealed that small aged animals harbored the higher infection rates of 65.45% and 62.86% in lambs and kids respectively. The represented data find out higher incidence of coccidiosis during the wet seasons of the year 56.8%. In the present study different *Eimeria* species was recognized, 9 species from sheep and 5 species from goats. It has been conducted that coccidiosis was a moderately common intestinal pathogen among sheep and goats in the study area.

Keywords: Sub clinical coccidiosis, small ruminants, Sulaymaniyah, Iraq

Introduction

Coccidiosis is caused by intestinal protozoan parasites belong to the genus *Eimeria* (1). It is considered an economically important disease, in Asia, Africa, Europe and America (2, 3). Coccidiosis can reduce the overall production and predispose animals to secondary diseases, with high mortality among young stressed animals (4).

Protozoan species of the genus *Eimeria* (Apicomplexa: Eimeriidae) are associated with intestinal infection (5, 6). Mixed infection with pathogenic and / or non-pathogenic species can happen at any time during animal life, however younger animals being more susceptible (7, 8). While the adult animals act as reservoirs for infection (9).

Goats become infected with 17 *Eimeria* species, *E. caprina*, *E. christensenii*, *E. arloingi*, and *ninakohlyakimovae* have significant pathogenetic effects (10). although 15 *Eimeria* species have been described to be parasitized sheep (11).

Among various species *E. ovina*, *E. ovinoidalis*, *E. ahasta* and *E. crandallis* are pathogenic and associated with symptomatic coccidiosis (12). The life cycle of *Eimeria* species passes in two phases. The endogenous phase when the parasites go through several divisions inside the intestinal cells, after ingestion of sporulated oocysts and sporozoites released in the intestinal lumen, and the exogenous phase happens outside the host's body in the environment (6). It required two to four weeks depending on *Eimeria* species and

environmental conditions (13). Animals become infected subsequent to the ingestion of sporulated oocysts. The sporozoites are released in the small intestine, invaded the epithelial cells of intestine. Finally, the oocysts released in the feces of infected host (14).

Eimeria species usually affect small intestine and the colon of the host, leading to histopathological changes like damage to the epithelial cell surface and atrophy of the villi connected to the first generation of schizonts, as well as destruction or hyperplasia of the crypts that accompany the formation of gamonts (15). The lesions cause decrease in the adsorptive surface of the intestine, subsequently interfering with adequate protein absorption and reduction in electrolyte absorption including important elements that required for normal growth and healthy appearance of lamb and kids (16).

The apparent clinical signs of coccidiosis are diarrhea, the infected animals demonstrate weakness, reduction in weight gain and rough hair coat (17, 18). In adult animals it characterized by hemorrhagic diarrhea (19). In kids or lambs, it characterized by watery diarrhea with clumps of mucus, However, rarely the color of feces changed to yellow or brown (10).

Morbidity with *Eimeria* infection might be 10-40% and the mortality may reach 10% (20, 21). Infection becomes more serious, especially when animals are reared in closed breeding system, predominantly when small aged animals held in overcrowded house or

irrigated pasture during winter months (21). In small ruminants' subclinical coccidiosis is common condition (7), and associated with reduced growth rate due to the depressed appetite and reduced in nutrient absorption that attributed to the damage to the intestinal surface (22). Conventionally, diagnosis of coccidiosis based on microscopic examination of fecal samples to find out *Eimeria* oocysts (23).

Although time and effort are the main limitations incorporate this technique, which depends on the skill and experience of the examiner (21). Morphological identification can be done by performing an effective flotation technique method using a saturated saline solution (24).

Molecular techniques (PCR) are applied for detection and differentiation of *Eimeria* species. However, PCR application for diagnosis of coccidiosis is associated with difficulties such as the wall nature of *Eimeria* oocysts, which is quite strong, additionally during subclinical cases when fewer oocysts are released. The DNA concentration obtained might be too low; consequently, the effective DNA amounts extracted from the oocysts are not essential for reliable PCR amplification and *Eimeria* detection (24).

To determine the species distribution, it's important to perform proper identification of coccidia species, due to the adverse effects of subclinical coccidiosis on animal health and productivity (25 , 6). Achieving a study is essential to find out the prevalence of subclinical coccidiosis based on the morphological identification of various

Eimeria species infecting sheep and goats in Sulaymaniyah province, and to clarify the impact of risk factors like age and seasons on the frequency of infection.

Materials And Methods

The study was carried out during the period between March 2021 and February 2022. In Sulaymaniyah province northern region of Iraq. Sheep and goats aged more than six months were selected randomly from different traditional small ruminants' farms belonging to four districts.

Fecal samples were collected from sheep (152) and goats (108). Animals were grouped according to age in age groups of lambs and adults for sheep, while kids and adults for goats. Regarding the season, according to the geographical distribution of Sulaymaniyah province, approximately rainy season is started from November till April, and from May till October considered as dry months of the year.

Samples were collected directly from the rectum of the animals using disposable gloves. Transport to the Microbiology laboratory, Veterinary Medicine College, Sulaimani University, and preserved in 10% formalin until processing

Coprological examination has been applied to all fecal samples for detection of *Eimeria* oocysts, using floatation technique by saturated saline solution with high specific gravity (26). Identification of *Eimeria* species was established using the morphological features of oocysts (shape, color, presence or absence of micropyle and

its cup) and stieda body under 400X magnifications (18).

Statistical Analysis

Analysis was performed for the obtained data using SPSS software V22, Chi-square test was applied to evaluate the association between coccidiosis and the expected risk factors, the value $P < 0.05$ was statistically significant.

Results

Based on the parasitological examination of the total 260 fecal samples, the results showed that 51.15% of the animals carried *Eimeria* oocysts (Table 1).

The infection rate of subclinical coccidiosis was higher among reared animals in Bakrajo region 53.85%. However lower frequency rate of 46.67% was reported in Qaradagh rejoin.

The study data revealed that oocysts of *Eimeria* species were significant differences in fecal samples of sheep (52.63%) and goats' (49.07%).

Subclinical coccidiosis represented a higher infection rate among small aged animals in lambs 65.45% and kids 62.86% with

significant difference ($P < 0.05$). The results denoted higher chance for infection was found during the wet seasons 57.60% than the dry months of the year 45.19%, statistically significant differences. Co-infection with more than one *Eimeria* species was common in both sheep and goats, among the examined sheep 58.62% were found to harbor single *Eimeria* species and 60.00% were infected with more than one species of *Eimeria*, in goats 39.66% of the goats were infected with one *Eimeria* spp. and 40.00% of them co-infected with more than one species (Table 2), although no significant differences were noticed between them.

Based on morphological characters of observed oocyst, identification of the total of nine *Eimeria* species oocysts from sheep *E. ovina*, *E. ahasta*, *E. faueri*, *E. intricata*, *E. weybridgensis*, *E. parva*, *E. ovinidalis*, *E. crandallis*, and *E. pallida*, and five types of *Eimeria* oocysts from goats *E. caprina*, *E. arlongi*, *E. hirci*, *E. christenseni* and *E. apsheronica* was identified.

Table 1. Epidemiological aspects of Subclinical coccidiosis of small ruminants in Sulaymaniyah province

Determinants	Variables	Total examined no.	No. of positive	Infection rate	X2 (p-value)	OR ** [95% CI]	Relative risk *** [95% CI]
Area	Tanjaro	75	39	52.00			
	Bakrajo	65	35	53.85	0.699		
	Sharazoor	60	31	51.67	[0.87]		
	Qaradagh	60	28	46.67			
Animal	Sheep	152	80	52.63	0.319	1.3	1.07
	Goat	108	53	49.07	[0.57]	[0.8- 2.2]	[0.8-1.3]
Age	lambs	55	36	65.45	4.092	2.2	1.44
	adult sheep	97	44	45.36	[0.43*]	[1.1-4.5]	[1.0-1.9]
	kids	35	22	62.86	3.936	2.2	1.48
	adult goats	73	31	42.47	[0.47*]	[1.0- 5.2]	[1.0-2.1]
Season	Wet months	125	72	57.60	4.003	1.6	1.27
	Dry months	135	61	45.19	[0.45*]	[1.0-2.6]	[1.0-1.6]
Total		260	133	51.15			

Note: - *Significant difference, **OR: odds ratio, ***CI: confidence intervals

Table 2. Pattern of *Eimeria* species infection in small ruminants from Sulaimaniyah province.

Patterns of infection	Sheep		Goat	
	No. of infected/ total	%	No. of infected/ total	%
Single infection	35/58	58.62	23/58	39.66
Mixed infection	45/75	60.00	30/75	40.00
Total	80	52.63%	53	49.07

Table 3. Frequency of *Eimeria* species recovered from sheep and goats.

<i>Eimeria</i> spp.	Animal	No. positive (%)	Single infection No. positive (%)	Mixed infection No. positive (%)	CI 95%*
<i>E. ahasta</i>	Sheep N=80	12	5	7	[9.025 – 9.975]
<i>E. ovina</i>		13	7	6	
<i>E. crandallis</i>		10	3	7	
<i>E. ovinidalis</i>		10	4	6	
<i>E. intricata</i>		5	2	3	
<i>E. weybridgensis</i>		6	3	3	
<i>E. pallida</i>		8	4	4	
<i>E. faurei</i>		6	3	3	
<i>E. parva</i>		10	4	6	
<i>E. caprina</i>	Goat N=53	12	5	7	
<i>E. arlongi</i>		14	8	6	
<i>E. christenseni</i>		9	3	6	
<i>E. apsheronica</i>		7	2	5	
<i>E. hirci</i>		11	5	6	
Total		133(51.15%)	58 (43.61%)	75 (56.39%)	

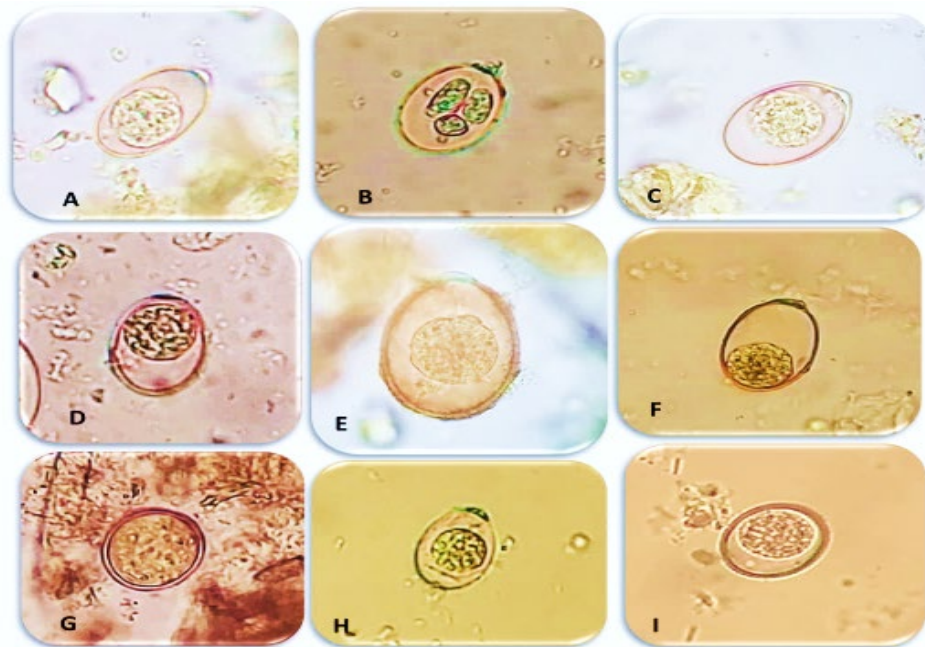


Fig. 1. Oocysts from *Eimeria* spp. recovered in sheep fecal samples from Sulaymaniyah province. Un sporulated oocyst of (A) *E. ovina*, (B) *E. ahasta* (sporulated oocyst), un sporulated oocyst (C) *E. faurei*, (D) *E. weybridgensis*, (E) *E. intricata*, (F) *E. ovinidalis*, (G) *E. parva*, (H) *E. crandallis*, and (I) *E. pallida*.(x 400 magnification).

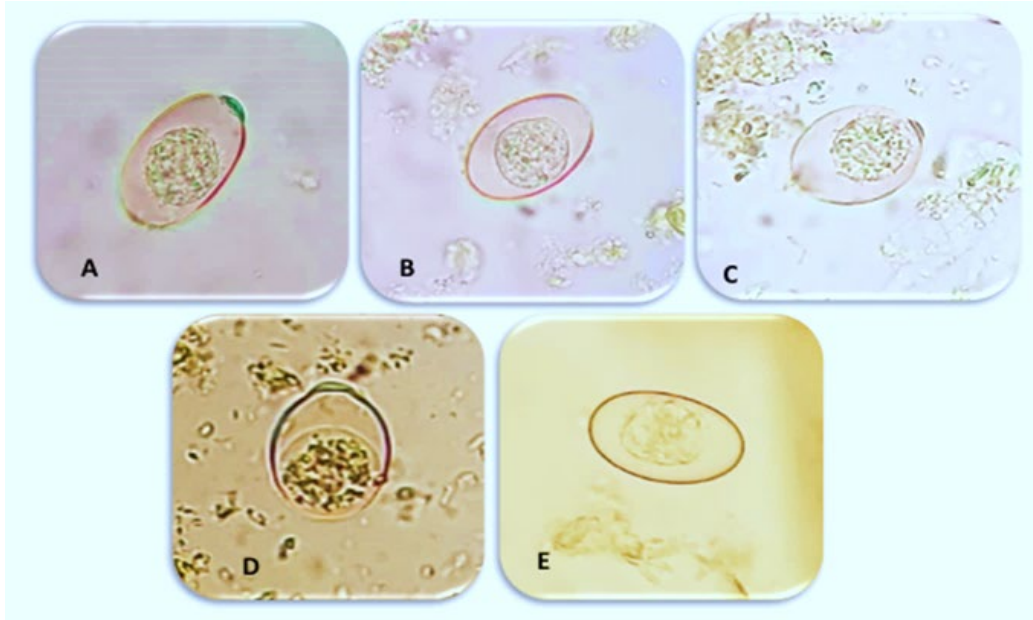


Fig.2. Oocysts from *Eimeria* spp. recovered in goat fecal samples from Sulaymaniyah province. Unsporulated oocyst (A) *E. arlongi*, (B) *E. caprina*, (C) *E. hirci*, (D) *E. christenseni*, and (E) *E. apsheronica*. (x 400 magnification).

Discussion

Small ruminant husbandry has a significant role in agricultural sector. Infection by *Eimeria* species in sheep and goats have been observed with various prevalence rates in different parts of the world. The overall infection rate of subclinical coccidiosis in current study among the examined animals was 51.15%. The current study indicated a decrease in the coccidiosis prevalence than the previous estimate 72% in sheep from Iraq Sulaymaniyah province (27).

The study data represented that the examined sheep 52.63% were parasitized by *Eimeria* species. In goats, oocysts of *Eimeria* spp. were found in 49.07% of the examined fecal samples. This finding is in agreement with previous study by Shaheed

and Al-Azizz (28) they have been found that 45.6% and 18.38% of the studied sheep and goats were infected with coccidiosis, respectively.

Contrary to the current finding the reported data by (29) revealed higher prevalence of 50.5% in goats than in sheep 37.5%, moreover (30) reported subclinical coccidiosis 69.05% of goats and 56.25% of sheep. Similarly, 83.6% of goats, and 60.9% of sheep were recorded by (31). In addition, the findings of (32) and (6) disagreed with current data, as they reported 73.91% and 60% in goats and 66.18% and 57.70% in sheep respectively.

Environmental factors as well as stress condition can trigger coccidiosis and results

in variation in prevalence rate in different geographical areas as well as between countries. The previous studied reported various prevalence rates, and higher infection rates of coccidiosis in sheep compared with present report. Higher infection rates of coccidiosis were 82.03%, 68.4%, 58% and 57.5% in Brazil, Egypt, Ethiopia and Iraq respectively (33- 36). However, low prevalence of 43.7% and 20.33% were reported from Ethiopia and Turkey respectively (29;37).

In goats, *Eimeria* spp. oocysts were reported with higher prevalence rates than the current study. Higher prevalence rates were in Vietnam 86.85%, India 90.96%, Mexico 60.29%, and in Myanmar 60.0% respectively (38;39;25;40).

The observed higher positive rates reflected poor hygienic conditions, that could be an enhancing measure for spreading of coccidiosis, such a condition permitting an increased amount of *Eimeria* oocysts in the environment, as a result occurring of food and water contamination consequently animal exposing to infection (41). Furthermore, high-density animal rearing system enabled the distribution of coccidia species during a short period of time (42).

Mixed infection with more than one *Eimeria* spp. is common under nature infection. Current data indicated that sheep 58.62% were parasitized by single *Eimeria* sp., and oocysts of more than one *Eimeria* spp. were found in fecal samples of 60.00%, goats also suffering from single and mixed infections 39.55% and 40.00%, respectively.

Similarly higher prevalence of mixed infection by different *Eimeria* spp. 89.3% and 73.0% in sheep reported by (36 , 34)

respectively, in addition (6) found the same result in both sheep and goats. In different to the current data (31) reported that goats 74.5% have more frequently mixed infection than sheep 43.6%.

Subclinical coccidiosis occurrence according to the age represented higher infection rates among younger animals than adults in sheep and goats, which is contrary to the result data reported by (32). In an agreement to the current study (38, 40) were reported higher prevalence rates among kids 96.25% and 71.4% in compare to adult goats more than one year of age 77.17% and 55.4%. In addition, prevalence rates of 100% and 97.95% in kids less than one year recorded by (39) and (43) were higher than in adult goats 84.61% and 92.02%, respectively.

Otherwise, higher incidence of coccidiosis was 69% in lambs less than 6 months of age than aged animals 12-24 months 18.5% (44 ;45).

Several factors could be attributed for finding of maximum infection rate with coccidiosis during animals' life, presence of high number of parturient females, overcrowding and breeding might also be associated (32). The immunological state of the animals, change in the grazing area, and the rearing environment may all be contributed factors to the incidence of parasites in different age groups (46).

It has been demonstrated that healthy sheep and goats are typically immune to the disease by the age of one year, however older animals can transmit diseases to younger ones (7, 47). Animals that have recovered from coccidia are mainly resistant to contracting re-infection by the same

species. however, immunity isn't absolute, and even oocysts of highly pathogenic species can be discovered in the feces of otherwise healthy animals (48). So, immunity to *Eimeria* spp. is specific but not solid, the presence of numerous oocysts may result in ongoing of reinfection, and raise the risk of environmental contamination, and livestock can contract both pathogenic and non-pathogenic *Eimeria* species at the same time (7). Although immunosuppression in old animals as a result of extreme stress might lead to clinical coccidiosis and oocyst shedding (49).

The current findings represented that infection rate of coccidiosis was higher during wet months

of the year than during the dry months, which is consistent with the data reported by (28, 36, 44) from other regions of Iraq, they found that maximum prevalence was 30%, 85% and 45% during December, February and January, respectively.

The high incidence of coccidiosis during wet and rainy months of the year might be attributed to the fluctuation in the climate conditions that allow the survival of oocysts and encourage their sporulation (45), additionally, overcrowding of animals during the wet season led to a disruption in sanitation conditions and increased the chance of infection more than once (41).

However, higher infection rate of coccidiosis in goats during summer months than in winter (47, 50). Moreover, the rearing animal management system was identified to have an essential role in the incidence of *Eimeria* infection. It has been found there is a close association between technification and intensity of infection (32).

Subclinical condition with non-specific clinical signs makes difficulties for its diagnosis, as a result, the diagnosis must be confirmed though other indicators such as coprological tests, management practices, age, and climatic considerations (51).

Identification of *Eimeria* spp. have been reported around the world with different prevalence values. The study results represented that different species of *Eimeria* parasitized the intestine of examined animals. Fourteen types of oocysts belonging to different species of *Eimeria* parasites including pathogenic and non-pathogenic were found. Nine *Eimeria* spp. in sheep morphologically recognized. Concerning the fecal samples of examined goats' morphological identification was defined five types of *Eimeria* oocysts.

Morphological characterization of oocysts is well known as a practical and reliable means for the differentiation of *Eimeria* spp. (52). The current recognized *Eimeria* spp. have already been described in small ruminants, by other authors from different areas. In sheep based on morphological characters of observed oocyst similar species were found by (34 and 36) in Iraq, same species were also recognized by (44) in Turkey.

The identical oocysts to the current reported *Eimeria* spp. in goats were also found by (25) and (38) in Mexico and Vietnam, respectively. Furthermore, similar studied based on morphological identification of *Eimeria* species, current recognized species were also reported by (6, 31) in sheep and goats.

The collected samples from sheep and goats in the current study were all fully formed feces, with few of them composed of soft-

formed feces. Severe diarrhea was not seen in any of the examined animals. Previous studies clarify the existence of genetic resistance against gastrointestinal parasites and coccidiosis in indigenous small ruminants (40). In an agreement to the findings of (25) the pathogenic *Eimeria* spp. *E. ovina*, *E. ahasta*, *E. ovinidalis*, and *E. crandallis* have been found in the feces of examined sheep. While no clinical signs of coccidiosis were observed in any of examined animals. Similarly, the pathogenic species of *E. arlongi*, and *E. christenseni* were recovered from goat fecal sample with no obvious signs.

The protozoan parasites of genus *Eimeria* display a good example of parasitism/co-existence. The *Eimeria* species lives within its host without considerable pathogenicity, providing a balance between host and parasite, although the balance has been disturbed by numerous stress factors that make the parasite activated, and following multiplication the condition becomes a severe illness (53). Coccidiosis considered as a potential cause for producers' losses due to treatment costs, delay in growth performance and occasionally death of small animals less than 3 months (54, 55).

CONCLUSIONS

The study data conclude that coccidiosis is endemic among small ruminants with moderate prevalence, different *Eimeria* species infected sheep and goats, and various risk factor has its impact on prevalence rate. Although traditional identification based on the morphology of the oocyst is still useful.

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دراسة الكوكسيديا تحت السريري في المجترات الصغيرة في محافظة السليمانية، العراق

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الخلاصة

تحدث الإصابة بالكوكسيديا بسبب أنواع الأيميريا ذات الأهمية الاقتصادية الكبيرة التي تصيب العديد من الحيوانات بما في ذلك المجترات الصغيرة. هدفت الدراسة الحالية لتقدير حدوث خمج الايمرية في المجترات الصغيرة في محافظة السليمانية خلال الفترة من اذار ٢٠٢١ إلى كانون الثاني ٢٠٢٢. تم جمع ٢٦٠ عينة براز (١٥٢ غنم و ١٠٨ ماعز) من مزارع المجترات الصغيرة تنتمي إلى أربع مناطق. تم تطبيق تقنية تعويم البراز لفحص عينات البراز. كشف فحص البراز ان الكوكسيديا تطفلت على أمعاء ١٣٣ حيواناً بنسبة إصابة ٥١.١٥ % ، مثلت كل من الأغنام والماعز الكوكسيديا تحت السريري النسبة المؤوية للخمج ٥٢.٦٣ % و ٤٩.٠٧ % على التوالي. عدوى مشتركة مع أكثر من *Eimeria spp.* كانت سائدة ووجدت في ٦٠ % من الأغنام و ٤٠ % ماعز ، بينما سجلت الإصابة بنوع واحد في ٥٨.٦٢ % أغنام و ٣٩.٦٦ % ماعز. كان معدل الإصابة بالكوكسيديا تحت السريري أعلى بين الحيوانات في باكراجو ٥٣.٨٥ % ، وأقل في الفرداغ ٤٦.٦٧ % . أظهر تأثير العمر على معدل الانتشار أن الحيوانات صغيرة السن تأوي معدلات إصابة أعلى بنسبة ٤٥ . ٦٥ % و ٨٦ . ٦٢ % في الحملان والماعز على التوالي. تشير البيانات الممثلة إلى ارتفاع نسبة الإصابة بالكوكسيديا خلال المواسم الرطبة من العام بنسبة ٥٦.٨٠ % . خلال هذه الدراسة، تم التعرف على أنواع مختلفة من الأيميريا ، تسعة أنواع من الأغنام وخمسة أنواع من الماعز. استنتجت الدراسة أن الكوكسيديا مرض معوي شائع بشكل معتدل بين الأغنام والماعز في منطقة الدراسة.

الكلمات المفتاحية: الكوكسيديا تحت السريري، المجترات الصغيرة، السليمانية، العراق.