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The First Record of Ovine *Eimeria* Species (Apicomplexa) and their Prevalence in Aljabal Alakhdar, Libya

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ABSTRACT

Coccidiosis is an infectious disease that affects small ruminants, mainly sheep, causing significant economic losses for the industry worldwide. Although the global prevalence of ovine coccidia is high, there is limited information regarding the prevalence of ovine *Eimeria* in Libya. The current study aimed to identify *Eimeria* species and their prevalence in native sheep in the Aljabal Alakhdar governorate of eastern Libya. 103 fecal samples were collected and examined using flotation, modified McMaster, and sporulation techniques. The overall prevalence of *Eimeria* recovered from the samples was 92%, with 12 species recognised depending on their morphological characteristics. The most dominant species were *Eimeria ovinoidalis* and *Eimeria ahsata*, accounting for 33.98% and 31.07% respectively. All sheep ages and sexes exhibited the presence of coccidian oocytes; however, lambs had a higher infection rate than adult animals. Also, the current results showed no significant difference in the prevalence of this parasite across the different regions. While *Eimeria* infection affects sheep growth in the Aljabal Alakhdar region, good hygiene practices and control measures should be implemented for lamb-fattening herds.

Key words: Coccidiosis, *Eimeria*, Libya, Prevalence, Sheep.

INTRODUCTION

Aljabal Alakhdar municipality (The Green Mountain) is in northern-east Libya, the country's largest region and is well-known as the most naturally vegetated part of the country. This part of the country is characterised by a moderate climate with an average precipitation of 500mm annually along the coast of the Mediterranean Sea in North Africa. Therefore, the area has the most important population of small ruminants, more than six million heads, and sheep are the main red meat sources for the resident's consumption. These animals have an essential role in the nation's livestock production (milk and wool) and contribute to food security and the quality of life for the people (Mohammed et al. 2022; Alsanousi and Mohammed 2024).

Like other agricultural animals, sheep are susceptible to various diseases, particularly parasitic infections. These issues can arise in stressful conditions, such as overcrowded environments, intensive production systems, undernutrition, and during weaning. Such factors negatively impact both the welfare and productivity of the animals (Baberi et al. 2021).

Ovine coccidiosis is an intestinal parasitic infection caused by obligatory unicellular protozoa of the genus *Eimeria* (Apicomplexa: Eimeriidae), which are host-cell-specific protozoa. All sheep ages are susceptible to coccidia infection; lambs aged four weeks to less than a year are more vulnerable than older sheep. Adults often develop immunity to disease, but in some cases such as malnourishment, stress and poor hygiene, they may show signs of vulnerability (Taylor 2009; Delano et al. 2015). Furthermore, adult carriers of coccidia typically do not exhibit any symptoms and continue to excrete oocysts in their feces, which contaminate pasture and water supplies (Chartier and Paraud 2012; Rodrigues Fde et al. 2016).

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Eimeria spp. has a direct life cycle requiring only one host to become infected by ingesting sporulated oocysts, the parasite's infective stage. These intracellular protozoa can enter and destroy the host's epithelial cells of the small and large intestines. With these cells, *Eimeria* undergoes two phases of parasite reproduction, asexual and sexual multiplication, which develop and produce zygotes (unsporulated oocysts) (Taylor et al. 2015). Animals with Eimeriosis exhibit subclinical symptoms due to altered intestinal mucosa function, which reduces feed efficiency and causes poor weight gain, slow growth, and production loss. Clinical signs of the illness, such as diarrhoea that frequently contains blood or mucus, dehydration, decreased appetite, and weakness, can result in death if untreated in extreme circumstances (Lassen et al. 2013; Yan et al. 2021). The severity of coccidiosis depends on the infective dose, the species of *Eimeria*, the host-parasite immune response, and the environmental condition of the barn (Eckert et al. 1995; Mesa-Pineda et al. 2021). Therefore, mortality rates among lambs infected with coccidiosis may approach 10% (Molina and Ruiz 2019). Recovering animals often fail to produce sufficient amounts of meat, milk, and wool, impacting our resources and livelihoods (Liu et al. 2003; Court et al. 2010).

Sheep hosts 15 *Eimeria* spp. mainly described by size and many morphological features (Bangoura and Bardsley 2020). Mixed infections by different types of *Eimeria* are common in naturally infected livestock; however, most show mild or no clinical signs. In addition, some of these types are known to be responsible for outbreaks of clinical coccidiosis among young animals. *E. ovinoidalis* is the most highly pathogenic type, followed by *E. crandallis* (Olmos et al. 2020; Carneiro et al. 2022), *E. ahsata* (Baberi et al. 2021) and *E. ovina* (Dittmar et al. 2010; Mohamaden et al. 2018). Infection with *E. ovinoidalis* induces severe and potentially lethal enteritis as the first-generation schizonts (meronts) are detectable during necropsy. This intercellular phase is associated with diphtheroid to hemorrhagic jejunitis, while later developmental stages can lead to hemorrhagic typhlitis in massive infections (Gregory et al. 1989; Olmos et al. 2020).

In addition, an infection caused by *E. crandallis* causes subsequent infection by *E. ovinoidalis*, although this infection tends to be less severe in lambs, primarily affecting the cecum and colon (Gregory and Catchpole 1990). Furthermore, *E. ovina* may cause the formation of polyps in the small intestine due to localized replication of the later stages of the parasite (Gregory and Catchpole 1987). Several factors affect the pathogenicity and clinical consequences, including the infectious dose of the species, its ability to replicate, the inflammatory immune response, co-infection with other pathogens, and the associated treatment strategies and stressors. It is important to understand that not all *Eimeria* spp. found in ruminants are considered significant pathogens; some types can inhabit the host without causing major damage to the intestinal mucosa (Bangoura and Bardsley 2020).

Coccidiosis significantly impacts small ruminants, practically sheep, influencing global morbidity and mortality rates. However, no established studies have examined the prevalence and nature of coccidiosis affecting animals or the types of *Eimeria* present in the Aljabal Alakhdar region in eastern Libya. Consequently, this study aimed to identify *Eimeria* spp. and the prevalence of this protozoa in local sheep and determine how this parasite affects sheep of all ages and genders throughout multiple regions.

MATERIALS AND METHODS

Ethical approval

The chairman of the Al-Mukhtar committee reviewed and approved all procedures used in the current study, which has reference number NBC: 007.A. 25. 33.

Study area

All samples used in this study were collected from the different countryside of Aljabal Alakhdar region, located in north-eastern Libya at an elevation of about 600 meters above sea level. This area lies between the north Mediterranean coast and a dry semi-desert area to the south. This region is characterized by a mild Mediterranean climate, with an average annual precipitation of 400mm in winter and summer temperatures reaching 22°C. Therefore, the area is a vast fertile land that provides unique pasture resources for most of the year and supports considerable diversity in livestock including sheep, goats, cattle, and camels. These animals mainly depend on consuming the green grass in the first half of the year and the hay stock during the second half (Mohammed and Ibrahim 2022; Mohammed et al. 2022).

Collection of fecal samples and laboratory examinations

A total of 103 fresh fecal samples were collected directly from the rectums of sheep of various ages and genders. Most animals appeared healthy, while others exhibited mild to severe diarrhea. The samples were saved in labelled plastic containers and transported to the Parasitology lab of the Veterinary Medicine College, Omar Al-Mukhtar University, where they were stored at 4°C until examination. The flotation test was conducted to detect the oocysts' presence, recover them, and culture them in 2.5% potassium dichromate. The concentration-modified McMaster technique was applied for all samples to estimate the count of oocytes per gram of feces (OPG) (Roepstorff and Nansen 1998). *Eimeria* oocytes were identified based on the morphological characteristics previously described in the literature (Eckert et al. 1995; Florin-Christensen and Schnittger 2018).

Statistical Analysis

The measurements of oocytes were coded and entered into a Microsoft Excel worksheet program for Windows 2010. Statistical analysis was performed using SPSS software version 21. Descriptive statistics such as percentages were used to indicate the prevalence of *Eimeria* infection. All results were considered statistically significant at $P \leq 0.05$.

RESULTS

In this study, fecal samples of local sheep were obtained to investigate the prevalence of recovered *Eimeria* spp related to the regions. Coccidia oocysts were found in 92 of 103 fecal samples examined (89.32%) that were collected from six different locations.

Fig. 1: Microscopic images of sporulated and unsporulated *Eimeria* spp. oocysts in sheep feces in Aljabal Alakhdar governorate, Libya; Original pictures. A=sporulated oocysts of *E. ahsata*; B=unsporulated E. oocysts of *E. ahsata*; C=sporulated oocysts of *E. faurei*; D=unsporulated oocysts of *E. faurei*; E=sporulated oocysts of *E. ovinoidalis*; F=unsporulated oocysts of *E. ovinoidalis*; G=sporulated oocysts of *E. ovina*; H=unsporulated oocysts of *E. ovina*; I=sporulated oocysts of *E. weybridgensis*; J=unsporulated oocysts of *E. weybridgensis*; K=sporulated oocysts of *E. granulosa*; L=unsporulated oocysts of *E. granulosa*. Unstained. Scale bars=10 & 40µm.

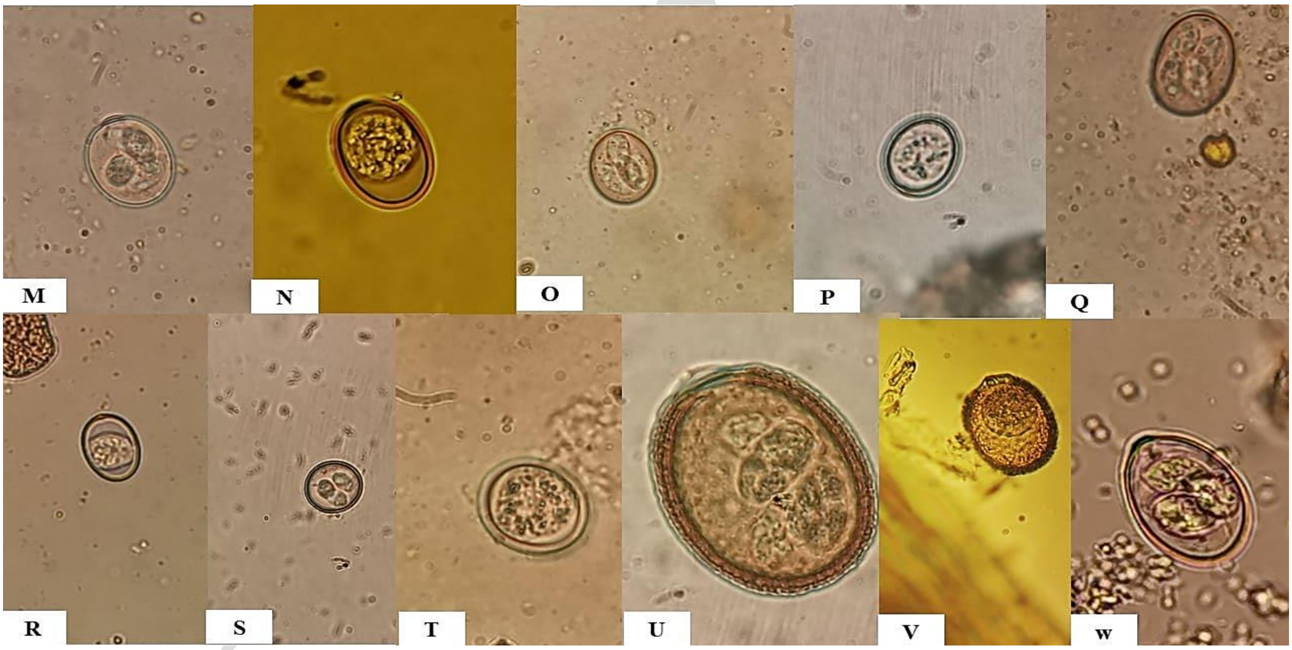

Fig. 2: Microscopic images of sporulated and unsporulated *Eimeria* spp. oocysts in sheep feces in Aljabal Alakhdar governorate, Libya; Original pictures. M=sporulated oocysts of *E. crandallis*; N=unsporulated E. oocysts of *E. crandallis*; O=sporulated oocysts of *E. pallida*; P=unsporulated oocysts of *E. pallida*; Q=sporulated oocysts of *E. marsica*; R=unsporulated oocysts of *E. marsica*; S=sporulated oocysts of *E. parva*; T=unsporulated oocysts of *E. parva*; U=sporulated oocysts of *E. intricata*; V=unsporulated oocysts of *E. intricata*; W=sporulated oocysts of *E. punctata* Unstained. Scale bars=10 & 40µm.

The current study identified 12 *Eimeria* spp. based on the morphological analysis of coccidia oocysts in the infected sheep (Fig. 1 and 2). The highest prevalence was detected for *E. ovinoidalis* (33.98%), followed *by E. ahsata* (31.07%), *E. granulosa* (29.13%), and *E. ovina* (28.16%). In contrast, the lowest prevalence was found for *E. weybridgensis* (3.88%) and *E. intricate* (1.94%) in all the samples (Table 1). In addition, *E. faurei* and *E. punctata* have the same prevalence (24.27%), as do *E. pallida* and *E. marsica* (13.59%). The other defined species were *E. crandallis* (14.56%) and *E. parva* (12.62%) (Table 1).

As shown in Table 2, the prevalence of recovered *Eimeria* oocysts was higher in lambs (91.3%) than in adult sheep (85.4%). Based on gender, statistical findings revealed a non-significant (P>0.05) association of the prevalence of *Eimeria* in males (95.8%) compared to

Table 1: The morphometric characteristic of *Eimeria* spp. in sheep

Species	Frequency n (%)	Form	Polar cap	Oocyst diameter (µm)	
				Polar	Equatorial
E. faurei	25(24.27)	Piriform to oval	Absent	29.79±2.91	22.2±2.27
E. ovinoidalis	35(33.98)	Ellipsoid	Absent	22.44±1.87	18.52±1.34
E. pallida	14(13.59)	Spherical	Absent	15.37±2.73	13.03±1.04
E. parva	13(12.62)	Spherical to Sub-spherical	Absent	16.63±1.22	16.63±1.22
E. marsica	14(13.59)	Ellipsoid	Absent	19.55±2.80	15.35±1.31
E. ahsata	32(31.07)	Ellipsoid	Present	32.89±2.97	22.25±3.54
E. ovina	29(28.16)	Ellipsoid	Present	28.70±1.26	18.65±1.35
E. granulosa	30(29.13)	Ellipsoid to Sub-spherical	Present	27.83±1.56	19.13±1.77
E. weybridgensis	4(3.88)	Ellipsoid to sub-spherical	Present	24.41±2.40	19.36±1.98
E. crandallis	15(14.56)	Sub-spherical	Present	21.52±1.25	18.33±2.10
E. punctata	25(24.27)	Ellipsoid to ovoid	Present	24.15±1.19	17.50±0.70
E. intricate	2(1.94)	Ellipsoid	Present	48.75±1.76	33.75±5.30

females (85.1%). While males are two times more likely to be diagnosed with coccidia infection than females, no significant differences were observed in the current study (P>0.05) (Table 2).

Table 2: The prevalence of recovered *Eimeria* spp. in sheep related to age and Gender

Parameters	Animals examined (n)	Positive No. (%)	COR	CL 95%
Age				
Lambs	62	57(91.3)	1.9	82.2-97.3
Adults	41	35(85.4)	Ref	
Gender				
Males	48	46(95.8)	4.02	85.7-99.5
Females	47	40(85.1)	Ref	

COR=crude odd ratio, CL 95%=confidence interval.

The highest prevalence was recorded in the Al mukhely region (97.6%), followed by El Bayda (92.3%), Omar Al Mukhtar (88.9%), Al Wesyta (87.5%), Al Quppa (75%), and Shahat (71.4%). However, there were no significant differences in the prevalence between target areas (P>0.05) (Table 3).

Table 3: The Prevalence of recovered *Eimeria* spp. in sheep based on regions

Regions	Examined sample no.	Positive (%)	COR	CL 95%
Al Quppa	16	12(75)	Ref	
Al Mukhely	41	40(97.6)	13.3	87.1-99.9
Omar AL Mukhtar	18	16(88.9)	2.6	65.3-98.6
Al Wesyta	8	7(87.5)	2.3	47.3-99.7
Shahat	7	5(71.4)	0.8	29.0-96.3
El Bayda	13	12(92.3)	4	64.0-99.8
Overall	103	92(89.32)		

COR= crude odd ratio, CL 95%=confidence interval.

DISCUSSION

Eimeria is the largest genus in the family Eimeriidae, with species that are both endogenous stages in the intestinal cells of their hosts and are also host-specific. Therefore, the distinction between *Eimeria* spp. generally relies on the biological and structural characteristics, morphology, and size of oocysts, as well as the type of the host (Eckert et al. 1995; Taylor et al. 2015). In the present investigation, the overall positive samples of *Eimeria* spp. infections were 89% and this estimated prevalence was higher than that reported in Iran (19.2%), Kenya (35%), Cameron (28.8%), India (54.68%), Egypt (60%), and Ethiopia (62.9%) (Yakhchali and Golami 2008; Kanyari et al. 2009; Ntonifor et al. 2013; Altaf and Hidayatu 2014; Mohamaden et al. 2018; Etsay et al. 2020). However, the current study's findings were lower in comparison to those observed in Turkey (100%), China (92.9%), and Nigeria (87.14%) (Kaya 2004; Wang et al. 2010; Sule et al. 2021). These variations in the prevalence rating could be attributable to breed, climate, and management systems such as stress, weaning, diet change, and sample size (Etsay et al. 2020; Carneiro et al. 2022).

Globally,15 intestinal and one abomasal (*E. arloingi*) *Eimeria* spp. have been observed in sheep (Wang et al. 2010; Ammar et al. 2019). Microscopically, twelve *Eimeria* spp. from all infected sheep were isolated in the present study depending on the morphometry of the oocysts. The results of this study were similar to Akyuz et al. (2019)'s work (12 types of *Eimeria*). In Egypt, a neighbouring country of Libya, some studies recorded 10, 11, and 14 ovine *Eimeria* types (Mohamaden et al. 2018; El-Alfy et al. 2020; Mohamed et al. 2022). Although the current work describes the same species consistent with these investigations, one type (*E. punctata*) defined in this work, didn't exist in the Egyptian studies. Similar types of sheep coccidia have been recorded worldwide for instance in Germany (12), China (12), Saudi Arabia (8), Iraq (10), Nigeria (9) and Brazil (10) (Dittmar et al. 2010; Wang et al. 2010; Ibrahim and Afsa 2013; Al-Rubaie and Al-Saadoon 2018; Sule et al. 2021; Carneiro et al. 2022). The variety of identified species between countries is due to location, the impact of climate, nutrition circumstances, age, breeds of animals, host immunity and stress caused by other diseases (Gashaw et al. 2020; Yunus 2023).

The most dominant type of isolated sheep *Eimeria* in this work was *E. ovinoidalis* (33.98%) which is a highly pathogenic species and is frequently seen in sheep around the world (Olmos et al. 2020; Al-Neama et al. 2021). Also, other pathogenic species, *E. ahsata* (31.07%) and *E. ovina* (28.16%) were frequently found as the second common type in the current finding. While, *E. crandallis*, has a moderate pathogenic effect, (Olmos et al. 2020), has the lowest prevalence in sheep (14.56%) compared to the other pathogenic types. Earlier investigations in different parts of the world commonly detect these pathogenic species in different ages and genders in sheep (El-Alfy et al. 2020; Olmos et al. 2020; Trejo-Huitron et al. 2020; Carneiro et al. 2022). The domination of these types of *Eimeria* could result from their high reproductive ability compared to other species (Carneiro et al. 2022). Numerous factors affect infections' pathogenicity and clinical outcomes, including the species, infection dose, replication potential, inflammatory immune response, concurrent infections with other pathogens, management practices, and stress. Coccidiosis can lead to acute and chronic damage, increasing susceptibility to secondary diseases. It is important to note that, not all *Eimeria* spp. in ruminants are significant pathogens; some can infect the host without causing major damage to the intestinal mucosa (Bangoura and Bardsley 2020).

The findings of this study revealed that all infected animals exhibited multiball infection with more than one or two of *Eimeria* spp., both pathogenic and non-pathogenic, which is similar to earlier results by Carrau et al. (2018) and Al-Neama et al. (2021). Mixed infections have been recorded in small ruminants, with over 80% of fecal samples from sheep containing two or more different species of coccidia (Wang et al. 2010; Carrau et al. 2018). The geographical region and weather conditions play significant roles in the ovine coccidia species, as these factors are associated with dry and semiarid areas (Souza et al. 2015; Al-Neama et al. 2021), such as the one observed in different regions of Al Jabal Alakhdar. The warm and humid environment creates a favourable condition for the rate and efficiency of oocyst sporulation, and this leads to increasing the potential for natural coccidian infection in sheep (Carrau et al. 2018).

The observation of the current work shows that the prevalence of recovered *Eimeria* spp. in lambs (91.3%) was higher than in adults (85.4%), which is comparable to results observed by Souza et al. (2015) and Carneiro et al. (2022). They documented that many factors play an important role in the high burden of infection in young animals, including a high density in the intensive system, seasons, and lack of hygienic-sanitary conditions. Ruminants can shed over a million oocysts per gram of feces during primary infections. Young animals are particularly susceptible to immunological factors, while adults acquire specific immunity to *Eimeria* species after their initial exposure. As a result, eimeriosis is considered a self-limiting illness. The frequency and intensity of oocyst shedding gradually increase, peaking around the time of weaning, and then declining in adulthood (Bangoura and Bardsley 2020). It is generally believed that adult animals have a limited role in the epidemiology of the disease, as older animals develop partial immunity to coccidia infection. This results in a noticeable decrease in the shedding of oocysts in their feces. In certain investigations, a major issue on commercial farms (fatting lambs) is the overcrowded transmission among the young lamb populations and the types of flooring used in flocks (Daugschies and Najdrowski 2005; Sufi et al. 2017).

The present finding found that male sheep were 4.02 times more likely to be exposed to *Eimeria* species than female sheep. Although COR suggested that males are more likely to contract the disease than females, there is no statistically significant correlation between gender and the prevalence of the disease. This data aligns with previous research indicating that gender is an important factor in the prevalence of coccidia. Male animals may be more susceptible to infections due to immunosuppression linked to higher levels of androgens, particularly testosterone, in their plasma during the reproductive season (Souza et al. 2015; Carneiro et al. 2022). However, prior studies found that rams were less likely than ewes to test positive for *Eimeria* infection. This may be due to the physiological condition of females and the larger number of samples examined (Mohamaden et al. 2018; Ramadan et al. 2018; Karimzadeh et al. 2022). Therefore, additional research is necessary to comprehensively understand the impact of gender on eimeriosis in sheep, as this insight may facilitate improved management and treatment approaches.

Regarding regions, the highest prevalence of recovered *Eimeria* spp. was recorded in Al Mukhely and El Bayda, with lambs showing greater susceptibility than adults. Also, the prevalence of the disease was related to the regions and age, with the highest prevalence recorded in the Al Mukhely region. This may be explained by the high stocking density of lambs in these areas as these regions are part of a mountain. It is believed that cold high places have an important role in increasing the shedding oocysts rate in sheep (Karimzadeh et al. 2022). Furthermore, naturally infected lambs often exhibit prolonged disease and excrete more oocysts when several *Eimeria* spp. are present (Catchpole et al. 1976).

This study reveals that not all sheep fattening farms use anticoccidial medications, either for preventive measures or for treating episodes of diarrhoea. Additionally, veterinary professionals in the local field show little interest in treating coccidiosis in sheep, leading to frequent misdiagnoses of the disease. However, it has been documented that controlling ruminant coccidiosis is more about achieving endemic stability and decreasing infection pressure to non-critical levels than eliminating the pathogen (Daugschies and Najdrowski 2005). It is not recommended to use a treatment approach for coccidiosis since once the oocysts are detected in the feces and coccidiosis is diagnosed, most of the internal parasite population has already completed its life cycle and intestinal damage has occurred. Animals with patent coccidiosis will take time to recover and experience protracted diarrhoea whether treated or not (Bangoura and Bardsley 2020).

Conclusion

To the author's best knowledge, this investigation confirms the presence and types of ovine *Eimeria* spp. in Aljabal Alakhdar province, eastern Libya for the first time. A high prevalence rate of coccidiosis was recorded in sheep in this area, as well as *Eimeria* spp. oocysts were observed in all ages with the lambs having the highest prevalence compared to adults. Furthermore, the present finding also documented the occurrence of a widespread mixture of natural infections with highly pathogenic and non-pathogenic species in sheep. Based on the results, the veterinary authorities are advised to include attention to regular surveys and follow-ups of the sheep flocks and adopt preventive programs for lamb fattening flocks to prevent a significant economic impact. Further research is also necessary to understand the financial burden associated with this parasite; these data will be beneficial in developing control programs and risk assessments of the infection.

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