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Prevalence of *Cryptosporidium* Infection in Small Ruminants in Four Northern Egyptian Governorates

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Abstract

Cryptosporidium SPP. considered as important gastrointestinal protozoan, that can be affect domestic animals including small ruminants. Data regarding the risk factors for infection with *Cryptosporidium* spp. in Egypt's small ruminants are scarce. Thus, the current study sought to ascertain the frequency of Cryptosporidium infection in sheep and goats in four Egyptian governorates as well as other risk factors. Using the modified Ziehl-Neelsen technique, 820 fresh faecal samples were collected from sheep and goats between January and December 2022 and analysed under a microscope to check for *Cryptosporidium* oocysts. Overall, 42.7% of animals were infected with *Cryptosporidium* spp., with 43% of sheep and 42.3% of goats having infection. Furthermore, locality, sex, age, and season are risk factors for small ruminant infection with *Cryptosporidium* spp., according to multivariate logistic regression study. There is a correlation between the prevalence of *Cryptosporidium* and a history of diarrhoea, especially in tiny ruminants that are part of big herds. Animals raised in the KafrElSheikh governorate, females, and calves with a history of diarrhoea were twice as likely to be infected with *Cryptosporidium* spp. Furthermore, animals raised in large herds, calves less than one year, and the winter season had three times the risk of contracting *Cryptosporidium* spp. Further research is required to determine risk factors and evaluate the significance of Cryptosporidium infection in Egyptian sheep and goats for veterinary public health.

Keywords: *Cryptosporidium*, Prevalence, Risk factors, Sheep, Goats, Egypt.

Introduction

Cryptosporidium is an important gastrointestinal protozoan belonging to A*picomplexa* phylum and family *Cryptosporidiidae* and affects wide range of animals and human [1, 2]. The first case of cryptosporidial infection was diagnosed in diarrheic lambs in 1987 by Rojo-Va'zquez *et al*. Later, the disease was recorded in sheep from various geographical areas throughout the world [3]. *Cryptosporidium* infects epithelial cells of the small intestine, as well as the stomach, liver, gall bladder, lungs and trachea of a variety of mammals including humans [4]. *Cryptosporidium* is primarily transmitted through the fecal-oral route and indirectly through contaminated food and water [5].

Cryptosporidiosis is a serious gastrointestinal disease that causes diarrhea and poor weight gain in sheep and goats [6], and it is more severe in young animals than in adults. The infected animals shed a considerable number of oocysts [7]. These oocysts

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are the main source of infection for both animals and humans. *C. parvum* was the predominant species found in pre-weaned diarrhoeal goat kids and sheep lambs [8], and among the ten species that were characterized, the latter showed infection with two *Cryptosporidium* spp., *C. ubiquitum* and *C. parvum* [9].

In small ruminants, the most typical clinical signs of a *Cryptosporidium* infection include lethargy, depression, abdominal pain, appetite loss, and, most significantly, diarrhoea, which is accompanied by a large number of oocysts being shed into the environment [10]. Additionally, cryptosporidiosis is a disease that is challenging to treat in many farm animals and is linked to high rates of morbidity and mortality in small ruminants, especially in the presence of other entero-pathogens. Due to animal death, growth retardation, veterinary care, and medicine, farmers consequently suffer large financial losses [11].

In Egypt, animals infected with *Cryptosporidium* spp. represent a substantial source of zoonotic infection as well as a potential economic loss. Ruminants have a reported prevalence of 32.2% for *Cryptosporidium* spp. [12]. Particularly, a high prevalence of infection with *Cryptosporidium* spp. oocyst was found in the fecal samples of neonatal calves (30.2%) [13] and neonatal lambs (30%) [14].

In light of this, a deeper comprehension of the risk factors linked to *Cryptosporidium* infection would be beneficial in order to comprehend the possible routes of transmission and the dissemination of disease inside farms. However, little is known about the risk factors associated to *Cryptosporidium* spp. infection in sheep and goats in Egypt generally, and details regarding this disease in the northern Egyptian governorates are scarce.

The present study set out to ascertain the prevalence rate and examine risk factors linked to the infection of sheep and goats with *Cryptosporidium* spp. in four governorates in northern Egypt.

Materials and Methods

Ethical statement

All of the study's methodology and procedures were approved by the ethical committee for animal experiments at Benha University (BUFVTM 10-12-2023). Sheep and goat owners provided their consent permission to examine their animals. All the animals subjected for the study received appropriate veterinarian care.

Study area

From January to December 2022, the study was conducted in four northern Egyptian governorates. The selected study governorates are Kafer ElSheikh, Qalyubia, Menofia, and Alexandria, which are situated in latitudes of 31°06′42″N, 30°56′45″E, 30°41°N, 31°E, 30°52°N, and 30°11′51″N, respectively, and longitudes of 30°56′45″E, 31.21°E, 30.99°E and 29°53′33″E, respectively, Fig. 1.

A subtropical desert climate (Classification: BWh) prevails at Kafr ElSheikh, Qalyubia and Menofia which are situated at Nile Delta of Egypt with an altitude ranging between 29.99-51.44 feet above sea level. The average yearly temperature in these governorates is 24°C, while the average annual precipitation is 2-5 mm. Alexandria is located at an elevation of 30.38 feet above sea level, has an annual temperature of 22.64^{o}C and 8.38 mm of precipitation. The main activities in these study areas are livestock rearing, agriculture activities and have large number of farms and pastures.

Animals and samples collection

Across-sectional study was conducted during January to December 2022 on sheep and goats raising in four northern Egyptian governorates to study the prevalence of *Cryptosporidium* spp. Thrus, field's formula [15] was used to calculate the sample size, with 50% predicted prevalence for goats and sheep [16], 5% precision, and a 95% confidence level. For each of sheep and goats, the minimal sample size was 384 based on precision of 5% with 95%CI and raised to 430 for sheep and 390 for goats in order to increase precision, respectively. The animals were selected randomly form different governorates under the study. From each animal, a rectum fecal sample was taken and put in a plastic bag for parasitological analysis. The samples were collected randomly for the four governorates under the study. Among the 820 fecal samples collected, 160 were from young animals under one year old with diarrhea, whereas the remaining samples were from asymptomatic sheep and goats. A questionnaire was used to collect general data on the animals investigated. Data included herd size, species, age, sex, season, contact with cattle, herd size and the presence of diarrhea.

Microscopic examination

The samples were maintained at 4 ^{o}C for less than 24 hours before being tested for *Cryptosporidium* oocysts in the laboratory. The fecal samples were analyzed using a modified of Faust's centrifuge-flotation method. Briefly, three grammes of fecal material were homogenized in distilled water and centrifuged for one minute at 1500 rpm. After removing the supernatant, the homogenization and centrifugation steps were repeated. Then, the supernatant discarded, and the sediment was centrifuged after being resuspended in $ZnSO_4$ 44%. After that, the supernatant was removed, and the sediment was examined for *Cryptosporidium* oocysts using modified Ziehl-Neelsen staining method [17]. The sample was microscopically examined under 100X to identify the oocyst based on morphological characters.

Statistical analysis

The statistical analysis of the questionnaire and fecal examination data was carried out using SPSS, version 24 (SPSS Corp., IBM, USA). The prevalence of *Cryptosporidium* spp. infection in goats and sheep was assessed using descriptive statistics. The Pearson's Chi-squared test was performed to compare the infection frequencies between groups. Bivariate and multivariate analyses were used to assess the relationship between *Cryptosporidium* spp. infection and variables. The potential risk factors with $P< 0.2$ (χ2-test) were taken into account while creating the final multiple logistic regression model [18-21]. In order to determine the 95% CI and odds ratio, a P value of less than 0.05 was deemed statistically significant. Utilising the Hosmer and Lemeshow goodness-of-fit test, the final model's fit was assessed.

Results

In sheep and goats, the total prevalence of *Cryptosporidium* spp. infection was 42.7% (350/820; 95% CI: 39.4-46.1). The prevalence rate was non-significantly (P=0.836) higher in sheep than goats, where sheep having a prevalence of 43.0% (185/430; 95% CI: 38.4-47.7) and goats having a prevalence of 42.3% (165/390; 95% CI: 37.5-47.3). In addition, the *Cryptosporidium* spp. infection was significantly (P<0.0001) prevalent in governorates situated in Nile Delta than Alexandria governorates.

According to the data analysis, sex and age had a significant (P<0.05) effect on the prevalence of *Cryptosporidium* spp. infection. The prevalence rate of *Cryptosporidium* spp. was seen in 28.5% of males and 46% of females, respectively. In comparison to other age groups, the prevalence rate was significantly (P=0.013) higher (50%) in young animals under one year old, Table 1.

Moreover, the seasonal variation in the studied areas had significant (P<0.0001) effect on prevalence of *Cryptosporidium* spp. in sheep and goats, where autumn had the lowest prevalence rate (28.3%) and winter (56.7%) had the highest prevalence rate, Table 1.

The prevalence of *Cryptosporidium* spp. was significantly (P<0.0001) associated with herd size, with a high prevalence rate of (56%) in large herds compared to a small one (33.3%). In addition, significant association (P=0.003) was found between *Cryptosporidium* infection and diarrhea in examined sheep and goats during the current study. Diarrheic animals had a higher prevalence than non-diarrheic ones, Table 1.

A multivariate logistic regression analysis revealed that the animal's sex and age, season, herd size, history of diarrhea, and location were all risk factors for increased *Cryptosporidium* spp. infection, Table 2.

A higher risk of *Cryptosporidium* spp. infection occurred in females (OR = 2.1, 95% CI: 1.4-3.3, P = 0.001) than in males raising in kafr Elsheikh (OR = 2.2, 95% CI: 2 -3.6, P =0.037), Menofia (OR =3, 95% CI: 2.3-4.1, P =0.003) and Alexandria (OR =3.6, 95% CI: 3.1-5, P < 0.0001) compared with those raising in Qalyubia governorate, Table 2. Moreover, the risk of *Cryptosporidium* spp. infection increased significantly in young animals below one year old (OR =2.8, 95% CI: 2.1-3.4, P =0.007), during winter season (OR =3.3, 95% CI: 2.1-5.1, P < 0.0001), particularly in animals with history of diarrhea (OR =2.4, 95% CI: 2.1-3.3, P < 0.002) and raising in large herd (OR =2.6, 95% CI: 2.2-3.6, P < 0.0001), Table 2.

Discussion

Cryptosporidium infects a wide variety of farm animals and humans, resulting in significant economic losses and significant public health concerns. It causes digestive problems, particularly diarrhea in neonates [22, 23]. According to this study, the shedding of *Cryptosporidium* oocysts is a common occurrence in sheep and goats in Egypt. Furthermore, as demonstrated in earlier studies, *Cryptosporidium* spp. should be considered one of the primary agents causing diarrhea and mortality in sheep and goats particularly newborns in Egypt [12, 24, 25].

The results of this study demonstrated that sheep and goats were susceptible to infection with *Cryptosporidium* spp. and sheep had a higher prevalence rate than goats, with 43% and 42.3%, respectively. This rate was lower than the previous reported rates in Mexican sheep and goats, which were 67.5% and 72.5 percent, respectively [4]. In another study in Egypt, Abd-El-Wahed [26] reported prevalence rate of 48.3% for *Cryptosporidium* in lambs.

Cryptosporidium spp. infect sheep and goats all over the world at varying rates. *Cryptosporidium* spp. prevalence rates in sheep were (31-59%) in Spain [27, 28], (10.1%) in Poland [29], (1.8%) in India [17], (2.5%) in Egypt [24], (11.2%) in Tunisia [30], (11.3%) in Iran [31], (13.3%) in Iraq [32], (14%) in west Indies [33] and (2.2%) in Papua New Guinea [34].

Furthermore, the prevalence of *Cryptosporidium* infection in goats in Spain was 30% [28], 17.7% in Iraq [32], 0% in Poland [29], (22.3%) in west Indies [33] and 4.4% in Papua New Guinea [34].

However, comparing the prevalence of *Cryptosporidium* spp. infection in livestock between different countries should be done with caution because matching for animal characteristics and raising conditions is difficult [4, 20, 35]. These differences in prevalence could be attributed to sample size, geographical region, climate, animal

age, diagnostic procedures employed, breed, hygiene conditions, and management practices [22].

Furthermore, contrary to previous findings [36, 37], we discovered a substantial relationship between sex and *Cryptosporidium* prevalence. This result come in accordance with Maurya, Rakesh, Pradeep, Kumar, Kundu, Garg, Ram, Kumar and Banerjee [17], where they found significant difference in prevalence of between male and females of small ruminants. This independent relationship between sex and *Cryptosporidium* prevalence may be due to differences in animals' susceptibility to the etiological agent [3].

Age is the most important risk factor in the incidence of *Cryptosporidium* among diverse species, with younger animals suffering the most morbidity. The present findings revealed that the prevalence of *Cryptosporidium* spp. infection was significantly correlated with age, where the prevalence rate was increased two times in animals below one year when compared to elder animals. This is consistent with findings of previous studies [38, 39]. In addition, These study's findings are consistent with those of Asmelash, Mesfin, Addisu, Aklilu, Biruk and Tesfaye [40] and Gharekhani, Heidari and Youssefi [31], who found the highest prevalence in sheep below one year and the lowest observed among older sheep over two years of age. Indeed, it appears that young animals are more susceptible to infection than adults [41, 42]. However, previous studies reported non-significant association between age of sheep or goats and prevalence of *Cryptosporidium* spp. infection [4, 43]. The highest occurrence rate in neonatal animals could be attributed to their undeveloped immune systems and high vulnerability to *Cryptosporidium* [44]. The difference could be due to differences in the presence of oocysts in the environment, *Cryptosporidium* infectivity, animal husbandry conditions, and grazing system [29].

The season-based prevalence was computed to determine the period of year with the greatest prevalence of *Cryptosporidium* infection in sheep and goats. Winter (56.7%) and spring (45.2%) were the seasons with the largest seasonal prevalence, while summer had the lowest prevalence (35.8%), followed by autumn (28.3%). This finding was consistent with previous studies of Morsy, Megeed, Hammam, Seliem, Khalil and Aboelsoued [45] and Agrawal, Shukla and Pande [46], they observed that the *Cryptosporidium* infection increased significantly in high rainfall and humidity weather. In contrast to our findings, Khan, Saleem, Durrani, Ahmad, Hassan, Shafee, Khan, Khan, Zaman and Khan [37] observed a significant prevalence of *Cryptosporidium* throughout the summer season in Pakistan, which could be linked to the country's natural environment and considerable rainfall during the summer.

This could be attributed to *Cryptosporidium* is a watery infection [47], it is more common during the wetter months of the year. The relationship between rainfall and waterborne disease demonstrates the reason of *Cryptosporidium* outbreaks during the winter where the viability of pathogens affected mainly by climatic change as temperature changing and heavy rainfall [48, 49].

There was a strong association between fecal consistency and the presence of *Cryptosporidium*, with diarrheic fecal samples having a greater prevalence one time more than formed fecal samples. This was in agreement with previous findings of Majeed, et al., [39] but contradict to findings of Romero-Salas et al., [4]. The higher *Cryptosporidium* spp. oocysts in the environment with high concentration considered as the more infectious source for the susceptible animals. Therefore, it is logical to assume that the presence of large amounts of oocysts along with the persistence of diarrhoea in newborns, which can continue up to two weeks, presents a risk of infection with Cryptosporidium spp. in small ruminants [10]. The infection spreads quickly in polluted environments through direct contact between animals [27].

Contrary to findings of [39], the results of present study showed strong association between herd size and *Cryptosporidium* spp. infection, where animals raising in the large herd (>30) contracted the infection three times more than other animals. This could be due to overcrowding in huge hard, which is a reasonable cause of increased contamination of the environment and, as a result, the risk of infection [50]. In addition, in the line of previous study of Romero-Salas et al. [4], we found no correlation between *Cryptosporidium* prevalence and contact with cattle.

Limitation of the present study is the sampling technique and diagnostic methods because the *Cryptosporidium* oocysts are intermittent shedding in infected animals and the microscopic examination has poor sensitivity and did not reliable to detect the oocysts in all affected animals. In addition, absence of molecular analysis in this study limited the value of the epidemiological data of the present study.

Conclusion

The current study found that *Cryptosporidium* is a common and ubiquitous parasite in sheep and goats in Egypt. Young animals, particularly those with diarrhea consider as source for infection with *Cryptosporidium* species, and hence better management and hygiene practices are required to prevent the protozoan's spread. The results of the present study revealed that locality, sex, season, and herd size were identified as risk factors associated

with *Cryptosporidium* infection in small ruminants. Further studies are necessary to determine other possible risk factors for small ruminant infections with *Cryptosporidium* in order to develop and put into practice efficient preventive hygiene measures against cryptosporidiosis in small ruminant farms.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article

Competing interests

There are no conflicts of interest declared by the authors.

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Authors' contributions

Conceptualization, methodology, formal analysis, investigation, resources, data curation, writing-original draft preparation, A.S., O.A.A., M.H.W., M.M., M.S. and A.A.; writing-review and editing, A.S., M.M., O.A.A., M.H.W., M.S. and A.A.; project administration, M.M.; funding acquisition, A.S., M.M., O.A.A., M.H.W., M.S. and A.A. All authors have read and agreed to the published version of the manuscript.

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TABLE 1. Prevalence of *Cryptosporidium* spp. infection in small ruminants from Egypt

Variable	Total examined animals	No of positive	No of negative	% of positive	95% CI	Statistic
locality						
Kafr ElSheikh	200	80	120		33.5-46.9	
Qalyubia	190	55	135	28.9	23-35.8	
				4340.00.		$\chi2$=33.211 df=3 P<0.0001*
Menofia	220	95	125	2	36.9-49.8	
Alexandria	210	120	90	57.1	50.4-63.7	
Species						
Sheep	430	185	245	43	38.4-47.7	
Goats	390	165	225	42.3	37.5-47.3	$\chi2$=0.043 df=1 P=0.836
Sex						
Male	150	42	108	28.00	21.4-35.7	
Female	670	308	362	46	42.2-49.8	$\chi2$=16.178 df=1 P<0.0001*
Age						
<1	260	130	130	50	44-56	
1-2	265	101	164	38.1	32.5-44.1	
>2	295	119	176	40.3	35-46.03	$\chi2$=8.614 df=2 P=0.013*
Season						
Winter	240	136	104	56.7	50.3-62.8	
Spring	210	95	115	45.2	38.7-52	
Summer	190	68	122	35.8	29.3-42.4	$\chi2$=38.584 df=3 P<0.0001*
Autumn	180	51	129	28.3	22.3-35.3	
History of diarrhea						
Yes	160	85	75	53.1	45.4-60.7	
No	660	265	395	40.1	36.5-43.9	$\chi2$=8.860 df=1 P=0.003*
Herd size						
Small (<30)	510	170	340	33.3	29.4-37.5	
Large (>30)	310	180	130	58.1	52.5-63.4	$\chi2$=48.203 df=1 P<0.0001*
Contact with cattle						
Yes	500	220	280	44	39.7-48.4	
No	320	130	190	40.6	35.4-46.1	$\chi2$=0.908 df=1 P=0.341
Total	820	350	470	42.7	39.3-46.1	

*The results are significant at P<0.05

TABLE 2. Risk factors associated to *Cryptosporidium* spp. infection in small ruminants

Variable	B	S.E.	OR	95% CI for OR		P value
				Lower	Upper	
location						
Kafr ElSheikh	0.486	0.233	2.2	2.0	3.6	0.037
Menofia	0.674	0.227	3.0	2.3	4.1	0.003
Alexandria	1.168	0.228	3.6	3.1	5.0	<0.0001
Sex						
Female	0.754	0.217	2.1	2.0	3.3	0.001
Age						
<1	0.418	0.232	2.8	2.1	3.4	0.007
>2	0.262	0.191	2.5	2.7	3.9	0.022
Season						
Winter	1.188	0.226	3.3	2.1	5.1	<0.0001
Spring	0.840	0.231	2.3	1.5	3.6	<0.0001
Summer	0.257	0.237	2.3	2.2	3.6	0.003
History of diarrhea						
Yes	0.349	0.252	2.4	2.1	3.3	0.002
Herd size						
Large (>30)	0.959	0.159	2.6	2.2	3.6	<0.0001

B: Logistic regression coefficient, SE: Standard error, OR: Odds ratio, CI: Confidence interval

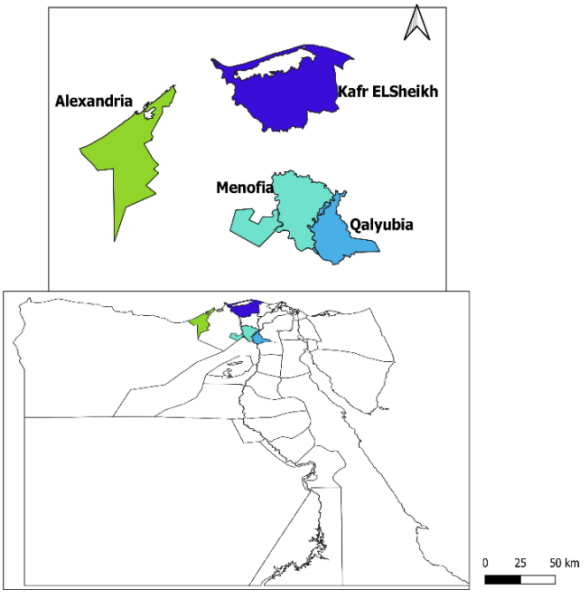

Fig. 1. Map demonesrate the governorates under the study (Map generated using QGIS software).

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معدلات الاصابه بالكريبتوسبوريديوم في المجترات الصغيره في اربع محافظات شمال مصر

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الملخص

طفيلي الابواغ الخبيئة يعتبر من الأوالي المعوية الهامة، ويمكن أن يؤثر على المجترات بما في ذلك الحيوانات المجترة الصغيرة. هناك ندرة في البيانات الوبائيه الخاصه بعوامل الخطر لطفيلي الابواغ الخبيئة غير متوفرة ونادره جدا وخصوصا في المجترات الصغيرة في مصر. ولذلك، هدفت الدراسة الحالية إلى تحديد معدلات انتشار وتقييم عوامل الخطورة المحتملة للخمج بطفيلي الابواغ الخبيئة في الأغنام والماعز في أربع محافظات مصرية. تم فحص 820 عينة براز من الأغنام والماعز مجهريا خلال الفترة من كانون الثانى الى كانون الاول 2022 للكشف عن اكياس بيض الطفيلي باستخدام تقنية زيل-نيلسن المعدلة. بلغت نسبة الإصابة 42.7%، حيث بلغت نسبة الانتشار بين الأغنام 43% والماعز 42.3%. بالإضافة إلى ذلك، كشف تحليل الانحدار اللوجستي متعدد المتغيرات أن المنطقة والجنس والعمر والموسم تعد عوامل خطورة للاصابه بطفيلي الابواغ الخبيئة في المجترات الصغيرة. وقد لوحظت العلاقة بين انتشار الطفيلي وتاريخ الإسهال خاصة بين المجترات الصغيرة وخصوصا الموجوده في قطعان كبيرة. كانت الإناث والعجول المصابة بالإسهال والحيوانات الموجودة بمحافظة كفر الشيخ أكثر عرضة للإصابة بطفيلي الابواغ الخبيئة بمقدار الضعف مقارنه بالحيوانات الأخرى. بالإضافة إلى ذلك، كانت العجول التي يقل عمرها عن عام وفي فصل الشتاء والحيوانات التي تربى في قطعان كبيرة أكثر عرضة للإصابة بطفيلي الابواغ الخبيئة بمقدار 3 مرات مقارنه بالحيوانات الأخرى. هناك حاجة إلى مزيد من الدراسات لتحديد عوامل الخطورة وتقييم أهمية الصحة العامة البيطرية للاصابة بطفيلي الابواغ الخبيئة في الأغنام والماعز في مصر.

الكلمات الدالة: الابواغ الخبيئة، نسبة انتشار، عوامل الخطورة، الأغنام، الماعز، مصر