

Parasite prevalence in soil samples from public areas in West Zawia, Libya

Iman Omar Ali Alkhammas^{1*}, Faraj Ali Mohammed Jalboub², Musab Salim Aboulqassim Madi³, Abdulkharim Abdu Saad Barkha⁴

^{1,2,3,4} Faculty of Veterinary Medicine and Agriculture, University of Zawia, Al-Ajeelat, Libya

*Corresponding author: a.alkhammas@zu.edu.ly

<https://orcid.org/0009-0000-0790-7498>

تاريخ الاستلام: 2026/04/03 تاريخ المراجعة 2026 /05/02 تاريخ القبول: 2026/05/15- تاريخ النشر: 2026 /06/06

Abstract

This study aimed to determine the prevalence of all parasitic forms (eggs, larvae, cysts, and oocysts) by using two flotation methods in soil from public places and fee-free gardens in West Zawia City, Libya. From 2025 to 2026, 160 soil samples were collected from various sites by simple random selection. To recover parasites, the soil samples were examined by sodium nitrate flotation, sucrose flotation method. The McNemar test and Kappa Index were used to analyses the statistical significance of the results.

The prevalence of soil parasites was as follows, *Toxocara* spp. eggs in sodium nitrate flotation (42.7%) and in sucrose flotation method (37%), *Iso spora* spp. in sodium nitrate flotation (14.7%) and in sucrose flotation method (22.7%), nematode larvae in sodium nitrate flotation (44.7%) and in sucrose flotation method (28%), *Eimeria* spp. in sodium nitrate flotation (12.7%) and in sucrose flotation method (28.7%), Coccidian oocyst and *Sarcocystis* spp. in sodium nitrate flotation (18%) and in sucrose flotation method (40%), *Dicrocoelium dendriticum* in sodium nitrate flotation (6.7%) and in sucrose flotation method (6%), Geohelminths in sodium nitrate flotation (10.7%) and in sucrose flotation method (7.4%).

Furthermore, following sucrose flotation method performance, modified Ziehl-Neelsen staining technique was done and oocysts of *Cryptosporidium* spp. was detected in 19 (14%) of soil samples. The McNemar test revealed significant differences between the sucrose and sodium nitrate flotation methods for parasite detection.

Key words: Parasites, flotation method, soil, prevalence West Zawia, Libya.

انتشار الطفيليات في عينات التربة من المناطق العامة في غرب الزاوية، ليبيا

ايمان عمر علي الخماس^{1*}، فرج علي محمد جلبوب²، مصعب سالم ابوالقاسم مادي³، عبد الكريم عبد السلام سعد بركة⁴

^{1,2,3,4} كلية الطب البيطري والزراعة، جامعة الزاوية، العجيلات، ليبيا

الملخص

هدفت هذه الدراسة إلى تحديد مدى انتشار جميع أشكال الطفيليات (البويض، واليرقات، والأكياس، والبويضات المتكيسة) باستخدام طريقتين للطفو في تربة من أماكن عامة وحدائق مجانية في مدينة الزاوية الغربية، ليبيا. خلال الفترة من 2025 إلى 2026، جُمعت 160 عينة تربة من مواقع مختلفة باستخدام طريقة الاختيار العشوائي البسيط. لاستخلاص الطفيليات، فُحصت عينات التربة باستخدام طريقة الطفو بنترات الصوديوم وطريقة الطفو بالسكرورز. استُخدم اختبار ماكنيمار ومؤشر كبا لتحليل الدلالة الإحصائية للنتائج.

كان انتشار طفيليات التربة كما يلي: بيض طفيليات التوكسوكارا (*Toxocara spp.*) في طريقة الطفو بنترات الصوديوم (42.7%) وفي طريقة الطفو بالسكرورز (37%)، وطفيليات الإيزوسبورا (*Isospora spp.*) في طريقة التعويم بنترات الصوديوم (14.7%) وفي طريقة التعويم بالسكرورز (22.7%)، يرقات الديدان الأسطوانية في طريقة التعويم بنترات الصوديوم (44.7%) وفي طريقة التعويم بالسكرورز (28%)، أنواع الإيميريا في طريقة التعويم بنترات الصوديوم (12.7%) وفي طريقة التعويم بالسكرورز (28.7%)، أكياس الكوكسيديا وأنواع الساركوسيسيتيس في طريقة التعويم بنترات الصوديوم (18%) وفي طريقة التعويم بالسكرورز (40%)، ديكروسيليوم ديندرينتيكوم في طريقة التعويم بنترات الصوديوم (6.7%) وفي طريقة التعويم بالسكرورز (6%)، الديدان المعوية في طريقة التعويم بنترات الصوديوم (10.7%) وفي طريقة التعويم بالسكرورز (7.4%).

علاوة على ذلك، بعد تطبيق طريقة التعويم بالسكرورز، تم استخدام تقنية تلوين زيل-نيلسن المعدلة، حيث تم الكشف عن بويضات طفيلية من جنس كريبتوسبورديوم في 19 عينة (14%) من عينات التربة. وأظهر اختبار ماكنيمار وجود فروق دالة إحصائية بين طريقتي التعويم بالسكرورز ونترات الصوديوم في الكشف عن الطفيليات.

الكلمات المفتاحية: الطفيليات، طريقة التعويم، التربة، الانتشار، غرب الزاوية، ليبيا.

INTRODUCTION

The broad category of parasites that reside in the soil during their development is known as soil-transmitted parasites (MandarinoPereira et al., 2010). One of the most significant risk factors for zoonotic parasite infection is soil contamination with parasite eggs, infectious larvae, cysts, and oocysts. The main parasites that may spread through soil are geohelminths (such as *Ascaris lumbricoides*, *Trichuris trichiura*, and hook worms) and zoonotic parasites (such as *Toxocara spp.*) (Waenlor and Wiwanitkit, 2007).

Previous research indicated that geohelminths were Africa's second leading cause of death for children under six (Ogbe et al., 2002). The larval stages of *Toxocara cati* and *Toxocara canis* are responsible for the zoonotic illness toxocariasis. These parasites cause the dangerous diseases known as visceral larva migrant (VLM) and ocular larva migrant (OLM). The primary sources of toxocariasis agents in urban areas are stray dogs and cats. For *Toxocara* eggs to become infectious, they must be incubated in soil for four to six weeks (Paul et al., 1988; Dubin et al., 1975).

The findings of soil investigation are influenced by a variety of variables, including sample collection times, parasite recovery techniques, sample volume and quantity, humidity, and soil desiccation (Nunes et al., 1994; Storey and Phillips, 1985).

Nonetheless, several investigations on the prevalence of parasites in soil samples have been conducted worldwide (Mandarino-Pereira et al., 2010; Rai et al., 2000; Uga et al., 1996). But,

there is a few epidemiological data on prevalence of parasites in the soil samples of various parts of Libya (Mohamed and Sirtiyah, 2023) study carry out in cities of western area and they focused mainly on animal parasite.

The aim of this study was to determine the prevalence of all parasitic forms (eggs, larvae, cysts and oocyst) by using two flotation methods in soil samples of public places and children's playgrounds in West Zawia City, Libya.

MATERIALS AND METHODS

Sampling

During 2025 to 2026, 160 soil samples were collected from various sites in West Zawia by simple random selection. At first, the town was geographically divided into five regions: north, south, east, west and center. Thirty two samples were collected from each region shown in figure(1). The study was focused on parks, public places and children's playgrounds. In each collection approximately 50 g was collected from 3 cm ground depth. As some samples were moist, thus all samples were air-dry at room temperature for approximately 24 h on tray.

Saturated sodium nitrate flotation

Isolation of eggs, oocysts and other parasitic forms was carried out for each sample by sodium nitrate flotation as described previously (Mizgajska-Wiktor, 2005) with some modifications. Briefly, the dried soil sample was mixed and sifted to remove solid objects. Then 20 g weighted sample was put into a 250 ml broad smooth opening Erlenmeyer's flask. Fifty milliliter of 5% sodium hydroxide (NaOH) (Merck, Germany) was poured into the sample and left for 1 h to separate eggs from the soil. Then, the sample was shaken for 20 min. Whole content of the flask was energetically poured into a 50 ml falcon tube. The sample was centrifuged for 3 min with 1500 rotations per minute (rpm) in order to settle the eggs and oocysts on the bottom. The supernatant was discarded and the sediment was washed three times with distilled water. After final washing the sediment was re-suspended in saturated sodium nitrate (NaNO₃) (Merck, Germany) with specific gravity 1.30 and centrifuged again (1500 rpm, 3 min). The tube was transferred into the stand, and the flotation fluid was added to the tube with a pipette until the fluid raised up to the brim of tube. Then on the surface of the fluid a 24 × 24 mm cover slip was placed and left for 30 min. During this time, parasitic eggs and oocysts stick to the glass. The cover slip with the hanging drop on the underside is placed on the slide and the specimen was prepared for microscopic observation.

Sucrose flotation method

Isolation of eggs, oocysts and other parasitic forms was performed for each sample by sucrose flotation method as described previously (Rai et al., 2000) with some modifications. Briefly, 4 g soil sample was dissolved in 50 ml distilled water and centrifuged in 1000 rpm for 5 min. Then the supernatant was discarded and sediment was re-suspended in 30 ml distilled water in a falcon tube and layered over with 15 ml sucrose (Merck, Germany) solutions with specific gravity of 1.40. After centrifugation at 800 × g for 5 min, the interface and the upper layer of

liquid was transferred to a new tube, and centrifuged at 1000 rpm for 5 min. The sediment was re-suspended in 50 ml distilled water and centrifuged at 5000 rpm for 5 min. The sediment was transferred to 1.5 ml micro tube and the trace of remaining sucrose was removed by two times washing with distilled water. The final sediment was used for direct microscopic examination.

Modified Ziehl-Neelsen staining

Cryptosporidium oocysts were identified by the sucrose flotation method followed by the modified Ziehl-Neelsen staining technique (John and Petri, 2006).

Data analysis

The McNemar chi-square test and Kappa Index were used for association and measuring the agreement between the results of each flotation method applied to recover parasites, respectively (K < 0.2 poor agreement, K 0.2 to 0.4 fair agreement, K 0.41 to 0.6 moderate agreement, K 0.61 to 0.8 good agreement, K 0.81 to 1.0 very good agreement) (Altman, 1992).



Fig 1–Map showing soil sampling sites in West Zawia city in Libya.

Results and discussion

Of the total 160 soil samples collected from five regions of in West Zawia City, 132 (80.2%) 119 (79.3%) were found to be positive for parasites. The prevalence of parasites in soil samples are summarized in Table 1. *Toxocara* spp. egg was more prevalent than others parasite in soil samples of West Zawia. *Cryptosporidium* spp. oocysts was detected in 18 (10%) of soil samples. In Table 1, we compared two methods for separation of parasites and prevalence of them, but we identified *Cryptosporidium* by sucrose method only. The McNemar test indicated that, there were statistically significant differences between results of two flotation methods in recovering *Toxocara* spp. eggs ($p = 0.006$), *Isospora* spp. oocyst ($p = 0.000$), Coccidian oocysts ($p = 0.000$), *Eimeria* spp. oocysts ($p = 0.000$) and nematode larvae ($p = 0.000$) in soil samples

(Table 1). Kappa coefficient indicated moderate or good agreement between two methods to recover the most of parasites.

This work was the first epidemiological study on prevalence of on all parasitic forms by using two flotation methods in soil samples of public places and children's playgrounds in West Zawia.

Table 1. Comparison of parasites prevalence detected by two flotation techniques in 160 soil samples in West Zawia public places.

| Parasite | Technique | | Statistical analysis | |
|--|--------------------------|-------------------|----------------------|-------------------|
| | Sodium nitrate flotation | Sucrose flotation | McNemar χ^2 | Kappa (STD error) |
| | N (%) | N (%) | (P value) | (K) |
| Helminths | | | | |
| <i>Toxocara</i> spp. eggs | 62 (42.7%) | 53 (37.0%) | 0.006 | 0.826 (0.048) |
| Geohelminth eggs | 19 (10.7%) | 14 (7.4%) | 0.063 | 0.651 (0.144) |
| <i>Dicrocoelium</i> eggs | 13 (6.7%) | 7 (6.0%) | 1.000 | 0.854 (0.144) |
| Nematode Larvae | 66 (44.7%) | 39 (28.0%) | 0.000 | 0.601 (0.064) |
| Protozoa | | | | |
| <i>Eimeria</i> spp. oocyst | 17 (12.7%) | 41 (28.7%) | 0.000 | 0.449 (.086) |
| <i>Isospora</i> spp. oocyst | 19 (14.7%) | 33 (22.7%) | 0.000 | 0.684 (.083) |
| Coccidian oocyst and <i>Sarcocystis</i> spp. | 27 (18.0%) | 40(28.0%) | 0.000 | 0.722 (.065) |

The prevalence of *Toxocara* in our study was higher than earlier studies from Iran (Motazedian et al., 2006) in Shiraz and Khorram Abad (Zibaei et al., 2010). This fact might be due to different climate conditions or diagnostic methods that was used in our experiments. Eggs of *Toxocara* spp. are resistant to environmental conditions and can remain transmissible for several years in favorable condition.

Tiyo et al. (2008) also reported a high rate of *Toxocara* egg contamination in soil samples from public squares in southern Brazil. Our study shows that *Toxocara* spp. were the most common parasites in soils of public places in West Zawia, high prevalence of VLM/OLM caused by this parasite is expected, especially in children. However, other studies have shown that the seroprevalence of toxocariasis in this group is not remarkable (unpublished data). It may be due to lack of appropriate diagnostic methods or limited studies. Eggs of geohelminths including *Ascaris* spp., *Trichuris* spp. and hook worms need a period of time, outside the host body to develop and attain infective stage. Presence of these parasites in the environment can be a public

health indicator (Saathoff et al., 2002). Low prevalence of these parasites in our study (Table 1) indicates relative good environment hygiene. Furthermore, the use of human feces as fertilizer which was avoided could be a factor of low frequency of finding these parasites in parks and other parts of urban areas. Coccidian parasites, including *Isospora* spp., *Eimeria* spp. and others, have animal origins and are capable of contaminating the environment through feces of dogs, cats and birds. Contaminations of soil with Coccidian oocysts that may be belonging to *Hammondia* sp., *Neospora* sp. and *Toxoplasma gondii* are epidemiologically important as they may contribute in maintaining parasite cycle in nature and providing a source of infection for human or other animals. Although, research on prevalence of *Cryptosporidium* oocysts in soil samples was rarely found, it was confirmed from different soil types (Mawdsley et al., 1996). *Cryptosporidium* oocysts are resistance to environmental conditions, for example, oocysts could tolerate to low temperature, even -10°C and still be infective to human and animals (Fayer, and Leek, 1984). In our study *Cryptosporidium* oocysts isolated from soil samples which exhibited relatively high prevalence (10%) should indicate attention to public health sector.

Conclusion

Zoonotic soil-borne helminth contamination was detected in the soil environment of public places, indicating a high probability of spreading intestinal helminthiasis to people living in their vicinity. Surveillance of the occurrence of helminth infections should be conducted in areas where stray or semi-domesticated dogs and cats reside. Public awareness campaigns should also be implemented targeting people in the community to increase their knowledge and understanding about animal healthcare to prevent and control the spread of zoonotic infectious diseases. Further studies are recommended in Libya to assess soil-borne parasite transmission and to provide a clearer picture of the epidemiological situation of parasites in Libya.

REFERENCES

- Altman G (1992). Practical Statistics for Medical Research. Chapman and Hall, London.
- Dubin S, Segall S, Martindale J (1975). Contamination of soil in two city parks with canine nematode ova including *Toxocara canis*: a preliminary study. Am. J. Public Health, 65(11): 1242-1245.
- Fayer R, Leek RG (1984). The effects of reducing conditions, medium, pH, temperature, and time on in vitro excystation of *Cryptosporidium*. J. Protozool. 31(4): 567-569.
- John D, Petri W (2006). Examinations of stool specimens. In: Markell EK, Voge M, editors. Medical Parasitology. 9th Ed Saunders: Missouri, pp. 393-415.
- Mandarino-Pereira A, de Souza FS, Lopes CWG, Pereira MJS (2010). Prevalence of parasites in soil and dog feces according to diagnostic tests. Vet. Parasitol, 170(1-2): 176-181.
- Mawdsley JL, Brooks AE, Merry RJ (1996). Movement of the protozoan pathogen *Cryptosporidium parvum* through three contrasting soil types. Biol. Fertil. Soils, 21(1-2): 30-36.
- Mizgajska-Wiktor H (2005). Recommended method for recovery of *Toxocara* and other geohelminth eggs from soil. Wiad Parazytol. 51(1): 21-22.
- Mohamed, A. R. A., & Sirtiyah, A. M. A. (2023). A Field Study to Evaluate the Efficacy of Changing the Type of Anthelmintic on Nematodes in Sheep in the Western Area of Libya. *African Journal of Advanced Pure and Applied Sciences*, 200-205.

- Motazedian H, Mehrabani D, Tabatabaee SH, Pakniat A, Tavalali M (2006). Prevalence of helminth ova in soil samples from public places in Shiraz. *East Mediterr. Health J.* 12(5): 562-565.
- Nunes M C, Sinhorini I L, Ogassawara S (1994). Influence of soil texture in the recovery of *Toxocara canis* eggs by a flotation method. *Vet. Parasitol.* 53(3-4): 269-274.
- Ogbe MG, Edet E, Isichel NN (2002). Intestinal helminth infection in primary school children in areas of operation of shell petroleum development company of Nigeria (SPDC), western division in delta state. *Nig. J. Parasitol.* 23(1): 3-10.
- Paul AJ, Todd Jr. KS, Dipietro JA (1988). Environmental contamination by eggs of *Toxocara* species. *Vet. Parasitol.* 26(3-4): 339-342.
- Rai SK, Uga S, Ono K, Rai G, Matsumura T (2000). Contamination of soil with helminth parasite eggs in Nepal. *Southeast Asian J. Trop. Med. Public Health*, 31(2): 388-393.
- Hamron, A. M., Barakat, A. H., Qaed, H. M., & Emran, F. (2026). Evaluation of the antifungal efficacy of *Salvia officinalis* extract against some clinical oral candidiasis isolates: a comparative study with standard antifungals. *Al-Farooq Journal of Sciences*, 2(1), 1242-1252.
- Saathoff E, Olsen A, Kvalsvig JD, Geissler WP (2002). Geophagy and its association with geohelminth infection in rural schoolchildren from northern KwaZulu-Natal, South Africa. *Trans. R Soc. Trop. Med. Hyg.* 96(5): 485-490.
- Storey GW, Phillips RA (1985). The survival of parasite eggs throughout the soil profile. *Parasitology*, 91(3): 585-590.
- Tiyo R, Guedes TA, Falavigna DLM, Falavigna-Guilherme AL (2008). Seasonal contamination of public squares and lawns by parasites with zoonotic potential in southern Brazil. *J. Helminthol.* 82(1): 1-6.
- Uga S, Ohkawa H, Amin-Babjee SM, Rai SK (1996). Contamination of soil with parasite eggs in and around Kuala Lumpur, Malaysia. *Jpn. J. Trop. Med. Hyg.* 24: 125-127.
- Waenlor W, Wiwanitkit V (2007). Soil examination for soil-transmitted parasite: Importance and experience from Thailand. *J. Ped. Inf. Dis.* 2(1): 11-13.
- Zibaei M, Abdollahpour F, Birjandi M, Firoozeh F (2010). Soil contamination with *Toxocara* spp. eggs in the public parks from three areas of Khorram Abad, Iran. *Nepal Med. Coll. J.* 12(2): 63-65.