

Prevalence of Soil Transmitted Parasites (Geohelminths) and its Impact in Different Locations of Sasiga District of East Wollega Zone, Ethiopia

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Abstract: Geohelminths are soil transmitted parasites where immature stages (eggs) require a period of development or incubation in the soil before they become infective and are estimated to infect greater than 2 billion humans worldwide. This cross sectional study was conducted from October, 2019 to June, 2020 in randomly selected locations of Sasiga district of East Wollega Zone of Ethiopia to determine the prevalence of Geohelminth (load of soil-transmitted helminthes on the soil) and its impact and also to identify the species of soil transmitted parasites (Geohelminth) in the study area. A total of 384 soil samples were collected from different locations of the district like compound of Abattoir, Primary School, Private Houses and Market places. A 15 g of the top soil samples were collected three times weekly from the children playground, front and behind of classrooms of primary schools, abattoir, private homes and their toilet areas into clean universal bottle using clean sticker or spoon. Modified Sheather Solution, Simple and Zinc Sulfate floatation techniques were used to examine the samples. From the total of 384 soil samples and examined for the eggs of parasites, overall of 245 (63.8%) of soil samples were positive for the eggs of different species of soil transmitted parasites in the area. Different prevalence rates were recorded in four places as 32 (80%), 97 (39.6%), 73(29.8%) and 43 (17.6%) at Abattoir, Market places, Primary Schools and Private homes, respectively. This showed that abattoir is highly contaminated (80%) while Private homes was the lowest 43 (17.6%) contaminated areas with different species of parasites in which statistical analysis showed a significant difference between the soils transmitted parasites and locations ($P < 0.05$). Different species of parasites like *Toxocara canis* 49(20%), *Ascaris lumbricoides* 50(20.4%), *Hook worm* 41(16.7%), *Echinococcus multilocuris* 35(14.3%), *Trichuris vulvus* 25 (10.2%), *Strongyloides stercoralis* 23(9.4%) Mixed (*Hook worm*, *Trichuris vulvus*, *A. lumbricoides* 22(9%) were observed. The prevalence of soil transmitted parasites is high and suggests that soil transmitted parasites are important public health problems in the study area. Thus, improving sanitation facilities, promoting socioeconomic status, educating the community will be warranted in addition to understanding of the mode of transmission and methods of prevention of the parasitic infections.

Key words: Geohelminth • Prevalence • Sasiga • Soil

INTRODUCTION

Geohelminth (also known as intestinal helminths and soil-transmitted helminths) infections are caused by *Ascaris lumbricoides*, *Trichuris*, hook worm (*Ancylostoma duodenale* and *Necator americanus*) and *Strongyloides stercoralis* and are estimated to infect

greater than 2 billion humans worldwide [1] Geohelminths are soil transmitted parasites where immature stages (eggs) require a period of development or incubation in the soil before they become infective [2].

These infections are more prevalent in tropical and subtropical regions of the developing world where adequate water supply and sanitation are lacking.

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The major public health significance and economic impact of this group of helminths is hard to quantify, although the WHO has estimated that more than 1000 million people world-wide are infected with one or more of the major pathogenic species of human and that 39 million disability adjusted life years are attributed to these four nematodes [3, 4].

Geohelminth infections are most prevalent among poor populations living in warm, moist climates in tropical and subtropical regions of low- and middle-income countries (LMICs), where access to sanitation and clean water is limited. Infections are acquired by ingestion of fecally contaminated food and water or direct contact with contaminated soil. Individuals living in endemic areas are commonly infected with more than one Geohelminth parasites and infections are generally acquired after 9 months of age and may persist into adulthood through repeated infectious exposures [5].

The epidemiology of Geohelminth infections in endemic areas is likely to have been influenced by two factors: (1) the process of urbanization in which increasingly greater proportions of the population are living in urban or urbanized environments, where the transmission of infections is localized to areas without sanitary infrastructure; and (2) anthelmintic treatment programs using periodic treatments targeted at school-age children, the major reservoir of infection in endemic communities, have been implemented in many LMICs. Geohelminth infections are relatively rare in high-income countries (HICs) such as the United Kingdom, where the few reported infections are detected in migrants or long-term travelers to endemic areas [6].

Soil-transmitted helminth affects more than 2 billion people worldwide [7] Other than human-specific parasites; intestinal nematodes affecting dogs have a relevant health-risk impact for both animals and human beings. The importance of these pathogens is often minimized by veterinarians and the general public, although *Toxocara canis*, hookworms (i.e. *Ancylostoma* spp.) and whipworms (i.e. *Trichuris vulpis*) are the most relevant canine helminths in terms of geographic distribution and clinical importance [8, 9].

Human beings become infected by *T. canis* most commonly by ingesting embryonated eggs from the soil. Other sources of transmission with dog intestinal nematodes include ingestion of larvae resting in tissues of paratenic hosts, or hookworm larvae in contaminated soil, which can penetrate the skin of humans walking barefoot [10]. The presence of infective eggs or larvae in the environment has a crucial role among the different

routes of transmission of dog intestinal nematodes in both humans and animals. In fact, human beings become infected by canine *Toxocara* spp. and *Ancylostoma* spp. most frequently via contaminated soil [11].

Various researchers from different countries have reported a high rate of soil and grass contamination with infective parasitic elements in market places, butcher shop, recreational, public and urban areas, i.e. parks, green areas, bicycle paths, playgrounds, sandpits. Stray dogs and other pet animals may defecate in public streets and areas, thus contaminating the environment with parasites and favoring zoonotic transmission and re-infection for other animals [10, 12]. On the other hand, close-contact with a pet has been considered an unlikely risk of infection with intestinal parasites for humans because the strong adherence of eggs on the animal's fur, the relatively high number of eggs which should be ingested to establish an infection and the long time for the embryo nation (i.e. minimum 2 weeks) [11, 12]. Rather than a self-contamination (e.g. with self-grooming transmitting eggs from the peri-anal region to other parts of the body), dogs may pick up *Toxocara* eggs on their hair by the scent-rolling [13].

Rural communities are living with their pet animals without the knowledge of these animal handling or management. Though, the concept of Geohelminths which may impact animal and public health is mandatory. However, a little study have been conducted in different areas of the country and also no research has been done in this study area, still there is lack of information on environmental contamination by infected dog faeces and how to decrease dog faeces from public place of the study areas. Therefore, objectives of the study were to determine prevalence of Geohelminths and its impacts in Sasiga district and to identify the species of soil transmitted parasites (Geohelminth) in the study area.

MATERIALS AND METHODS

Description of the Study Area: East Wollega is one of the 18 Administrative Zones of Oromia National Regional State, Ethiopia. Administratively, the zone has 17 districts, of which 17 are rural districts which are again subdivided into 286 Peasant Associations (PAs). Nekemte Town, which is located at a distance of 331km from Addis, is the capital of the zone. Astronomically, it is located between 9°5' N latitudes and 3610' minute East longitudes. It is located in the western part of Oromia Region, bordered with Benishangul Gumuz Regional State in the western; west Wollega Zone in the West, Horo Guduru Wollega

Zone in the East and Buno Bedelle Zone in the Southwest, Jimma zone in the south. The land area of the Zone is estimated to be 1,315,894.65 hectare. Agro climatic zone of the area is High land (7.18%), Midland (51.08%) and Lowland (41.74%). It experiences tropical climate because of the relatively high angular position of the sun. The mean annual temperature is fairly high. Generally, mean annual temperature of the Zone varies from 10-36°C [14].

The annual rainfall pattern in the Zone generally decreases from East to West following the physiographic nature of the Zone. The mean annual rainfall of the Eastern high lands range from 1800-2400mm, while in the central plateaus range between 800-1800mm and in the remaining parts of the Zone it becomes between 800-1600 mm and becomes less than 1200 mm in the Southwestern parts of the Zone. As reported by [15]. Livestock population of East Wollega Zone is 2,282,100 Bovine; 533,267 Ovine; 35,230 caprine; 28,403 horses; 158, 635 donkey; 16,466 mule and 2,073,732 poultry [16].

Sasiga District: At present it has 32 peasant associations which are administrative sub divisions of which 27 are rural peasant associations and 5 administrative town center. Gelo is the district administrative center.

The district is located in the western part of East wollega Zone, Nekemte city at a distance of 17kms and 348kms from Addis Ababa. It is bounded by West Beni Shangun Gumuz north Guto Gida district in South Diga and Guto Gida has a total area of 97,31,9 hectar. Astronomically, the district is located between 9°10°-19°3° North latitude and 36°27°59° East longitudes. The mean minimum and maximum annual temperature ranges between 23°C and 32°C, respectively. The mean annual rain fall is 1200-1800 ml. It lies on altitudinal range of 1500m-1960m above sea level (a.s.l.). The district has high livestock potential with 121,800 cattle, 14,685 Ovine, 12,859 Caprine, 9,489 Equine, 87,655 Poultry; 100,871 people; 20700 school children and 6000 Dogs as reported by [17,18], respectively.

Study Samples: Soil samples from different localities like compound of primary schools, abattoir, private houses and market places in the study area were involved.

Study Design: A cross sectional study was conducted from October, 2019 to June, 2020 in randomly selected different localities of Sasiga district for soil samples (Compound of Abattoir, Primary School, Private Houses and Market places) to determine the load of soil-transmitted helminthes on the soil.

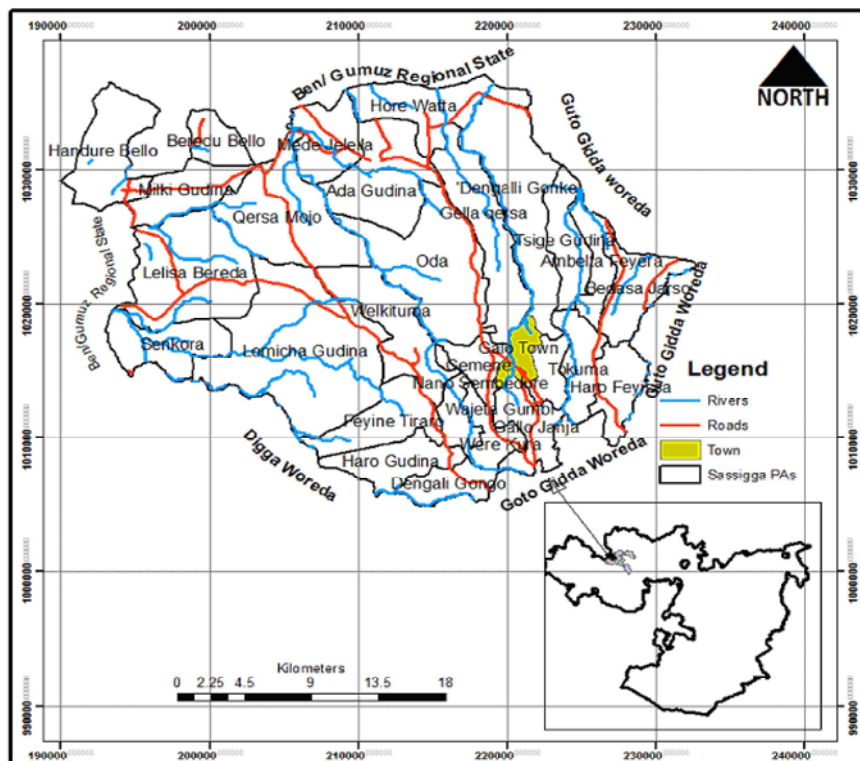


Fig. 1: The map of Sasiga district

Sample Size Determination: The local government was consulted on the purpose of the study and permission well as support was obtained to conduct the study. From the total 97,319 hectare land area or coverage of the district, primary schools, abattoir compound and market places which considered for the survey were covered by 3,400m², 400m² and 2500m² land areas, respectively. Soil samples were taken from primary school by using plot (m²) per soil samples in which children could play heartily such as school office and latrine areas were considered as the target areas. The same technique was used to collect soil samples from open market places, compound of abattoir and private homes. A [19] formula was used to calculate the desired sample sizes for this study.

$$n = \frac{(1.96)^2 \times P_{exp} (1-P_{exp})}{d^2}$$

where, n = required sample size, P_{exp} = expected prevalence, d = desired absolute precision

$$n = \frac{(1.96)^2 \times 0.5 (1-0.5)}{(0.05)^2} = 384$$

Accordingly, a total of 384 soil samples were collected to determine the load of soil-transmitted helminthes on the soil of primary schools, open market places, compound of abattoir and private homes.

Sample Collection and Transportation: Soil samples were collected three times weekly from different locations of target areas. 15g of the top soil (down to a depth of not more than 2cm) was collected from the playground, front and behind of classrooms of primary schools, private homes and their toilet areas into clean universal bottle using clean sticker or spoon [20]. The humidity was always favourable with the soil full of moisture according to [21]. So, a total of 384 soil samples were collected from different locations of the study areas using universal bottle and then transported to Wollega University Veterinary Laboratory Parasitology Room for examination.

Procedures of Soil Sample Examination

Samples Processing Procedures: The (15g) of top soil samples were collected in the morning hours from 6.00 am-11am, when the larvae and eggs of geohelminths were still active and fresh. This soil was mostly loamy soil, rich in organic manure or nutrients. The (10g) of soil samples were dissolved in 100ml tap water and Modified sheather solution flotation was prepared using 454g of sugar which

was dissolved in 355ml of warm tap water and then 10g of dissolved soil sample was suspended with 15ml-modified sheather solution filtered in white plastic bucket by using tea strainer, then drops to centrifugal tube and the mixture centrifuged at 2,000 rpm for 3-5minutes. Then the centrifugal tubes take off from centrifuge, shacked and stood up in rack up to form meniscus with cover slip for 2-4 minute. Then, the cover slip was gently left upward from test tube and immediately place on clean microscopic slide. The preparations should be examined or viewed under microscope for the parasites using, X10 and X40 objectives [22]. The ova/larvae of parasites are identified with reference to Atlas of Parasitology as reported by [2].

RESULTS

Load of Geohelminths in Soil Samples: Out of a total of 384 soil samples collected from different localities of the district and examined for the eggs of parasites, overall prevalence of 245 (63.8%) of them were positive for the eggs of soil transmitted parasites in the study area (Table 1). This soil transmitted helminth infections were recorded in four (4) randomly selected areas of the district namely, Abattoir 32 (80%), Market places 97 (39.6%), Primary Schools 73(29.8%) and Private Homes 43 (17.6%). The result of the screened soil samples from each location is indicated that, Abattoir is the highest (80%) while Private Homes were the lowest 43 (17.6%) contaminated areas with different species of soil transmitted parasites in the study areas. However, statistical analysis did show a significant difference among the results obtained in the four locations (P< 0.05).

Species of Soil Transmitted Parasites (Geohelminth) Identified: Out of soil samples collected from different places of the district to be examined for the presence of eggs of soil transmitted parasites, 245 (63.8%) of them were positive for eggs the parasites in the study area (Table 1). This soil transmitted helminth infections were recorded in four (4) randomly selected areas of the district namely, Abattoir 32 (80%), Market places 97 (39.6%), Primary Schools 73(29.8%) and Private Homes 43 (17.6%). All of the places considered for the examinations were contaminated with different species of soil transmitted parasites like *Toxocara canis* 49(20%), *Ascaris lumbricoides* 50(20.4), *Hook worm* 41(16.7), *Echinococcus multilocuris* 35(14.3), *Trichuris vulvus* 25 (10.2), *Strongyloides stercoli* 23(9.4) *Mixed (Hook worm, Trichuris vulvus, A. lumbricoides* 22(9%). Among the species of parasites observed during this study,

Load of Soil transmitted parasites in Soil samples of different locations of the area

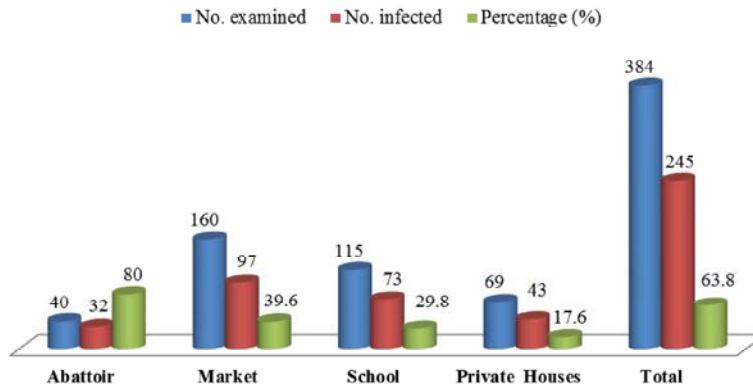


Fig. 2: Load of soil transmitted parasites (Geohelminth) in Soil Samples of different locations

Table 1: Eggs of species of soil transmitted parasites (Geohelminth) in different places

Sites of soil samples	Egg of Parasites observed							Total (%)
	<i>Toxocara canis</i> (%)	<i>A. lumbricoides</i> (%)	Hook worm (%)	<i>Echinococcus multilocur</i> (%)	<i>Trichuris (vulvus, trichiura)</i> (%)	<i>Strongyloides stercoli</i> (%)	Mixed (Hook worm, <i>Trichuris vulvus</i> , <i>Ascaris lumbricoides</i>)(%)	
Abattoir	10 (31.3)	5(15.6)	3(9.4)	4(12.5)	3(9.4)	5(15.6)	2(6.3)	32(80)
Market	21(21.7)	19(19.6)	18(18.6)	13(13.4)	12(12.4)	7(7.2)	7(7.2)	97(39.6)
School	11(15)	20(27.4)	13(17.8)	10(13.7)	3(4.1)	6(8.2)	10(13.7)	73(29.8)
Private Houses	7(16.3)	6(14)	7(16.3)	8(18.6)	7(16.3)	5(11.6)	3(8)	43(17.6)
Total	49(20)	50(20.4)	41(16.7)	35(14.3)	25(10.2)	23(9.4)	22(9)	245(63.8)

A.lumbricoides was slightly the leading geohelminth parasite which is followed by *Toxocara canis* (20%) in the areas. However, there is no statistical difference between the species of parasites and locations ($P>0.05$).

DISCUSSION

Soil-transmitted helminth (Geohelminths) infections are among the most common infections worldwide and affect the poorest and most deprived communities [23]. Geohelminth infections are most prevalent among poor populations living in warm, moist climates in tropical and subtropical regions of Low-and Middle-Income Countries (LMICs), where access to sanitation and clean water is limited. Infections are acquired by ingestion of fecally contaminated food and water or direct contact with contaminated soil. Individuals living in endemic areas are commonly infected with more than one geohelminth parasite and infections are generally acquired after 9 months of age and may persist into adulthood through repeated infectious exposures [5]. The study was conducted in Sasiga district of East Wollega Zone to determine the prevalence and species of geohelminth parasites in the study areas. The study comprised different localities of the district like compound of Abattoir, Primary Schools, Private Houses and Market places to determine the load of soil-transmitted helminthes

on the soil and its impacts in addition to identify the species of parasites (geohelminth) in the study areas.

Out of 384 examined, overall of 245 (63.8%) soil samples were positive for the eggs of different species of soil transmitted parasites in the study area. The result indicated that higher prevalence of Soil Transmitted Helminths (STH) was obtained in this present study as compared to different prevalence rates of Soil Transmitted Helminths (STH) infections on soil contamination with helminth eggs (7.9%-10.6%) reported by [24] and (18.6%)[25] from Poland. This is due to the presence of many stray or uncontrolled dogs having potential to contaminate the environment by their faeces in the study area. So, because of the lack of good environmental and personal hygiene and additionally, due to the poor or absence of fences around abattoir, primary schools and private households, the school children those plays without shoes (baring foot) may be vulnerable for the infection of Soil Transmitted Helminths (Table 1). It is also higher than the study conducted by [26] who reported 50.6% on soil contamination with geohelminth parasite from Gorgora Town, Northwest Ethiopia. [27] reported that 53.6% geohelminth egg content from the study environment of Nigeria which is lower than the current study. But the result of this finding is lower than previous studies conducted in Slovakia 79.2% [28] 79.3% in Iran [29] and in Brazil 78.6% [30].

Soil samples were collected from four different locations (Abattoir, Market places, Primary Schools and Private Homes) of the district where public activities might be considered as high. Different prevalence's of soil transmitted parasite's eggs were recorded in these four (4) randomly selected areas of the district as 80%, 39.6%, 29.8% and 17.6% in Abattoir, Market places, Primary Schools and Private Homes, respectively. Analysis of the result indicated that, the highest numbers of different species of soil transmitted parasite's eggs (80%) were recorded in Abattoir compound.

This might be due to the compound (Abattoir) attracts different species of canids those have potential to contaminate the compound by their faeces. ²⁷ reported higher prevalence's of eggs/larvae in soil 70%, 70%, 43.3% and 37% with respect to the different sample sites like back of toilet, behind classroom block, playground and front of classroom, respectively. But, majority of these sample sites were primary school does not have toilet facilities which had the highest prevalence of geohelminths in the environment. The result of the screened soil samples from Private Homes were the lowest 43 (17.6%) contaminated areas in which different species of parasites were observed. However, statistical analysis did show a significant difference among the results obtained in the four locations ($P < 0.05$).

Overall of 245 (63.8%) of soil samples were positive for eggs of six different species of soil transmitted helminth at different locations of the district. The observed eggs of these parasites were *Toxocara canis* 49(20%), *Ascaris lumbricoides* 50(20.4%), *Hook worm* 41(16.7%), *Echinococcus multilocuris* 35(14.3%), *Trichuris vulvus* 25 (10.2%), *Strongyloides stercoli* 23(9.4%) and mixed (*Hook worm*, *Trichuris vulvus*, *A. lumbricoides* 22(9%). Among the species of parasites observed during this study, *A. lumbricoides* was slightly the leading geohelminth parasite in the areas. However, there is no statistical difference between the species of parasites and locations ($P > 0.05$). This might be due to the stray dogs defecate everywhere in the field of the study areas. Additionally, they said that the public squares, sandpits, playgrounds, beaches are always at a high risk for heavy contamination by pet faeces and public parks and green areas are always contaminated by parasites of dogs. Also prevalence ranged from 17.8 to 87% Ogun State, South West Nigeria, which was ascribed to the high biotic potential of the worm as well as the ability of eggs to with stand adverse conditions [31].

The result is in line with the finding of [2, 5] who reported *Ascaris lumbricoides*, *Trichuris trichiura*, *Hook worm* and *Strongyloides stercoralis* as the commonest and well known parasites. The most common STHs which are found worldwide are: *Ascaris lumbricoides*, *Trichuris trichiura* and the hookworms, [32] with the greatest public health burden occurring in developing countries, particularly in sub-Saharan Africa [33] Scholars have indicated as eggs of *Toxocara* spp., eggs and larvae of *Ancylostoma* spp. and eggs of *T. vulpis* have been found from soil and faecal samples in public areas from Europe, the Americas, Africa and Asia [34]. The result is also supported by study of [32] from Kaduna, Nigeria who reported the prevalence (62.3%) of parasites. [35] reported prevalences of different species of parasites including *Ascaris lumbricoides* (12.5%) in which children were positive for double infections (*Ascaris lumbricoides* and *hookworm*).

Ascaris lumbricoides (20.4%) was slightly the dominant soil transmitted parasite (geohelminth) observed during this finding. As indicated by Smyth [36] eggs of *Ascaris* have an inner shell layer of lipoprotein nature which makes them more resistant to harsh environmental. *Ascaris* is cosmopolitan and its distribution is largely determined by local habits in the disposal of faeces, because its eggs reach the soil in human faeces and so contaminate the human environment [37]. The parasite is one of the major public health problems in communities where the prevailing social environment is characterized by poverty, poor housing, inadequate sanitary practices and overcrowding [3, 38].

Toxocara canis is the second dominant (20%) next to the *Ascaris lumbricoides* (20.4%) during this study. Eggs of *T. canis* may be present on the hair of both stray and privately owned dogs, with the latter considered as a more important risk for human infection due to the frequent contact with people [10, 34] indicated as human beings become infected by *T. canis* most commonly by ingesting embryonated eggs from the soil. Other sources of transmission with dog intestinal nematodes include ingestion of larvae resting in tissues of paratenic hosts, or hookworm larvae in contaminated soil, which can penetrate the skin of humans walking barefoot.

In contrary, *Hook worm* 41(16.7%) was observed during this study as compared to the finding of [27] who indicated *Hookworm* ova showed the highest occurrence in the soil sample followed by *Ascaris* ova and *Strongyloides* larvae in Ebenebe Town, Anambra State of Nigeria. Different scholars also showed *Hookworm filariform* larvae present in the soil infect a suitable host

by actively penetrating the skin (especially for *Ancylostoma* spp.) and/or via the oral route (*Ancylostoma* spp., *Uncinaria* spp) [39-43]. Eggs of the *Strongyloides stercoli* 23(9.4%) was the least soil transmitted parasite seen in the current study which is supported by finding reported by [44] from Kathmandu, Nepal. Also *S. stercoralis*-like larvae have been found in soil samples from Iran [45,46] Mixed or double infections (*Hook worm*, *Trichuris vulvus*, *A. lumbricoides* 22(9%) were also observed during this study. Similarly, mixed infections either by two or more geohelminths has also been reported by other investigators [47-49].

The egg of *Echinococcus multilocuris* 35(14.3%) was one of the soil transmitted parasite detected during the current study. The prevalence of hydatid cysts in harboring cattle, sheep, goats, camel were 35.15%, 11.78%, 4.9%, 16.79%, respectively. Humans can become infected if they ingest substances infected with *Echinococcus* eggs [50]. By this finding, 25 (10.2%) eggs of *Trichuris vulvus* were observed. Infection with this parasite is the third most common helminth infections of humans. The distribution of trichuriasis is worldwide, being most abundant in the warm moist regions of the world [51]. In Ethiopia there are 21 million people infected with this parasite, which accounts 13% of the disease burden in sub-Saharan Africa [52]. It is spread via fecal-oral transmission and high prevalence occurs in areas with tropical weather and poor sanitation practices [33]. The parasite commonly occurs together with *Ascaris lumbricoides* and likewise mainly affects children.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions: All locations (Abattoir, Market places, Primary Schools and Private Homes) of the Sasiga district considered for the study were positive for eggs of different species of soil transmitted parasites with highest record at Abattoir (80%). Different species of soil-transmitted helminths such as *Toxocara canis*, *Ascaris lumbricoides*, *Hook worm*, *Echinococcus multilocuris*, *Trichuris vulvus*, *Strongyloides stercoli* and mixed like *Hook worm*, *Trichuris vulvus*, *A.lumbricoides* were identified during this study. The present study revealed that a higher prevalence (63.8%) of soil-transmitted helminths was recorded in the district. So, the result of this finding implies that parasitic infections are important public health problems and the parasite has potential to affect thousands of people as a result of several risk factors in the areas with poor hygiene. The result also provides evidence for the high risk of acquiring STHs

infections from the contaminated soil in the study area. Having these points: the community should get public education (using Medias) and practice good hygienic standards which are now considered as a measurement to reduce the public health impact of these parasites; avoiding animal defecation in public areas or immediate collection of stool by the pet owner is crucial to reduce environmental contamination by dog's faeces; periodic anthelmintic treatments for all the community members and the animals should be practiced, especially for stray dogs; open field defecation by the children and other community members should use public latrine to prevent and control of the disease transmission and further investigations should be conducted to understand current status of these soil-transmitted helminths the infection.

Data Sharing Statement: The data collected and used to support this article can be offered by the first or corresponding author upon request.

Ethical Approval and Consent to Participate: Ethical approval (A formal letter) for this study was granted from Wollega University; Department of Veterinary Clinical Science (VCSc) and Laboratory Technology to Sasiga district Education office, Health Center and Livestock Resource, Development and Fishery Office that approved the request for the samples to be collected. All the participants were informed about the purpose and benefits of the study and verbal consent from each participant was obtained. Consents were taken in the presence of a third independent party and approved by the School of Veterinary Medicine.

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