A STUDY OF THE PREVALENCE AND ULTRASTRUCTURE OF ASCARIS LUMBRICOIDES INFECTING CHILDREN IN DAKAHLIA PROVINCE, EGYPT

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Abstract

Background: Ascaris lumbricoides is one of the most prevalent and largest nematodes found in humans is Ascaris lumbricoides. It is widespread throughout the world, affecting 25% of the world's population, and is most prevalent in tropical and subtropical regions. Some studies have been performed to describe the ultrastructure of the A. lumbricoides nematode using scanning electron microscopy (SEM).
Patients and Method: The current study was performed on 300 schoolchildren, (162 males and 138 females), aged (1–15 years) from both rural and urban areas at Dakahlia province, to detect A. lumbricoides (Type Egypt) in their faeces. The samples were collected with the approval of the Local Ethics Committee of Zagazig University, Faculty of Science. Written informed consent was obtained from the authorities of the Egyptian Ministry of Education and from students' parents under the supervision of a responsible consultant doctor in each school. The A. lumbricoides worms extracted from faecal samples of infected cases after macroscopic and microscopic examination were then examined by scanning electron microscopy (SEM).
Results: The intestinal parasitic infections with A. lumbricoides was positive in 42 cases (14%) of the studied cases. Mixed infection with two parasites, Ascaris lumbricoides and Enterobius vermicularis, in the same patient formed additional 42 cases (14%) of the examined cases. The frequency of infection was not associated with either age, or sex. SEM images described the criteria of different parts of studied A. lumbricoides (Type Egypt). The anterior end of A. lumbricoides revealed broad cervical alae and three large lips, one dorsal and two subventral, with the inner face of the free edge of each lip bearing an uninterrupted row of small, blunt, conoid denticles along almost its entire length. The denticles were almost of equal size and typically unicuspid.
Conclusion: Our survey conducted to study the distribution and abundance of A. lumbricoides nematodes among children at Dakahlia province. The prevention and control programs against sources and reservoirs of A. lumbricoides should be planned by public health based on reliable information from systematic surveillance. In addition, the ultrastructure study of adult A. lumbricoides (Type Egypt) worm by SEM gives more details about criteria of worm that help understanding the morphology in these species.

Keywords: Nematodes, Ascaris lumbricoides, Ultrastructure, SE

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1. INTRODUCTION

Ascaris lumbricoides (also known as roundworm) is a soil-transmitted nematode that causes ascariasis, which has affected the world's population for centuries. Annually, about 800 million to one billion people in the world are infected with Ascaris lumbricoides, and more than 60,000 people die from the disease (Shah and Shahidullah, 2018).
Ascariasis has been described in children and adults in tropical and subtropical areas with poor sanitation and personal hygiene and in places where human faeces are used as fertilizer. The disease is frequently documented in sub-Saharan Africa, Latin America, and East Asia. There is a higher risk of infection in non-endemic areas due to the increased rate of migration and travel (Claus et al., 2018).
A. lumbricoides is a large pink, or white worm. The adult female can reach up to 20 cm to 30 cm in length, and the adult male can reach up to 15 cm to 20 cm. The female worms are thicker and have a straight rear end. The male worm is slender, with a ventrally incurvated rear end and two retractile copulating spicules.
The life cycle of A. lumbricoides, is 12–18 months and involves five stages. An adult female produces about 200,000 eggs per day, which are shed along with faeces in un-embryonated, non-infective form. Embryonation occurs in soil at an optimum temperature of 20–25 °C with sufficient moisture and O_2. The infectious larva develops within the egg in about 3–6 weeks. The infection occurs beyond the ingestion of embryonated egg-

contaminated food and water. Larva is stimulated to hatch out by the presence of an alkaline pH in the small intestine and the solubilization of their outer layer by bile. Larva penetrates the intestinal wall and is carried to the liver through the portal circulation. Then it travels via blood to the heart and to the lungs via pulmonary circulation within 10–14 days of infection. In the lung, the larva molds twice, enlarge, and break into alveoli. From the alveoli, the larva then passes up through the bronchi and into the trachea, where it is swallowed. The larva passes down the esophagus to the stomach and again reach the small intestine. Within the intestine, the parasite molds twice and matures into an adult worm. Sexual maturation occurs within 6–10 weeks, and the mature female discharges its eggs in the intestinal lumen, which are excreted along with faeces, continuing the life cycle. The adult worms can live 1–2 years (CDC, 2019).

Patients with ascariasis may not experience any symptoms, only exhibiting long-term signs of undernutrition and growth retardation. The most frequent symptoms in symptomatic patients are anorexia, intermittent diarrhoea, abdominal pain, bloating, nausea, and vomiting. The Loeffler syndrome, which includes pneumonitis, eosinophilia, dyspnea, hemoptysis, and fever, can develop if there are a considerable number of larvae travelling through the lung. Small intestinal blockage, appendicitis, intussusception, cholecystitis, cholangitis, pancreatitis, and other conditions can develop as a result of adult worms migrating to tubular organs during superinfection, including the biliary and pancreatic systems (Olopade et al., 2018).

The best diagnostic test is still the stool exam for ova and worms. Eggs microscopically appeared tri-layered, large oval brown with a mamillated coat. As female worm can produce up to 200,000 eggs a day, this makes detection during the stool examination easier (Leung et al., 2020). Besides stool, sometimes the adult worm can be seen while coming out of the rectum, but it can also be coughed up or passed in the urine (Lamberton and Jourdan, 2015). Infected patients may present with eosinophilia that could be detected in the complete blood count during the active migration phase from the intestine to the lungs, and larva can be found in the sputum. Abdominal x-rays can be sensitive but not specific when a whirlpool sign exists. Ultrasound and CT scans can be used to identify worms in the gallbladder and biliary duct. Endoscopic retrograde cholangiopancreatography (ERCP) can also be used for diagnosis (Sundriyal et al., 2015).

This study aimed to collect and examine faecal samples from Egyptian schoolchildren in Dakahlia province, survey Ascaris lumbricoides in the collected samples. The study also aimed to examine the ultrastructure of Ascaris lumbricoides by scanning electron microscopy (SEM).

2. PATIENTS AND METHODS

This study conducted on school children from both rural and urban areas. The samples were collected after approval of Local Ethics Committee of Zagazig University, Faculty of Science. Written informed consent was obtained from authorities of Egyptian ministry of Education and from students' parents under supervision of responsible consultant doctor in each school.

The current study was performed on 300 schoolchildren, (162 males and 138 females), aged (1–15 years) from both rural and urban areas at Dakahlia province. From El Senbalawin, there were (41 males and 30 females), from Dekrnis (30 males and 26 females), from Agga (29 males and 24 females), from Belqas (34 males and 27 females) and from Nabroh (28 males and 31 females). 46% of the children with age (8 - 12 years), followed by 40% with age (4 - 8 years), 10% with < 4 years and 4% with age (12 - 15 years).

Conducted children were subjected to clinical examination from September 2017 to September 2018. Fresh stool samples were collected from each child in a clean, covered and labelled plastic container. All the specimens were immediately transferred to Zoology Department Laboratories, Faculty of Science, Zagazig University.

Faecal samples were collected in clean plastic containers, mothers were asked to handle the sample immediately after being passed stool sampled were examined macroscopically for the colour, odour, consistency and presence of mucous, blood or visible parasites. Samples were processed using Formalin ethyl-acetate concentration technique. After concentration a drop of sediment was placed onto a microscope slide and covered with a cover-slip. The entire cover-slip was examined with × 40 objective for Ascaris lumbricoides (Type Egypt).

The stool containing Ascaris lumbricoides samples were collected directly from host faeces, fixed in 4F1G solution and 2% Osmium tetroxide, transferred to 70% ethanol, dehydrated through a graded ethanol series solution, and washed before drying using the critical point drying technique. The samples were then mounted on metal stubs, sputtered with gold or palladium, and examined using SEM with a secondary electron detector at 20 KV. The SEM examination was carried out at the Faculty of Agriculture, Mansoura University (Tahmasebi et al., 2015).

3. RESULTS

Table 1 showed that 46% of cases had an age of 8–12 years, followed by 40% with an age of 4–8 years, 10% with less than 4 years, and 4% with an age of 12–15 years. As regards sex, there was a male predominance; about 54% of cases were males and 46% were females.

Table (1): Distribution of the studied cases according to age and sex.

Age (years)	*Incidence No. (%)*	*Sex*	
		Male ***No. (%)***	***Female*** ***No. (%)***
1 – 4	30 (10%)	16 (53.33%)	14 (46.67%)
4 – 8	120 (40%)	65 (54.16%)	55 (45.84%)
8 – 12	138 (46%)	74 (53.62%)	64 (46.38%)
12 - 15	12 (4%)	7 (58.33%)	5 (41.67%)
Total Number	**300 (100 %)**	162 (54%)	138 (46%)

Parents were mainly illiterate in 38% of cases, followed by being able to read and write in 26%, attending university in 22%, and having a diploma in 14%. Regarding residence, 60% were from rural areas and 40% were from urban areas, as shown in Figure 1. Figure 2 showed that the majority of cases got water from tap sources (70%), storage tanks (30%) and (62%) had accepted hygiene.

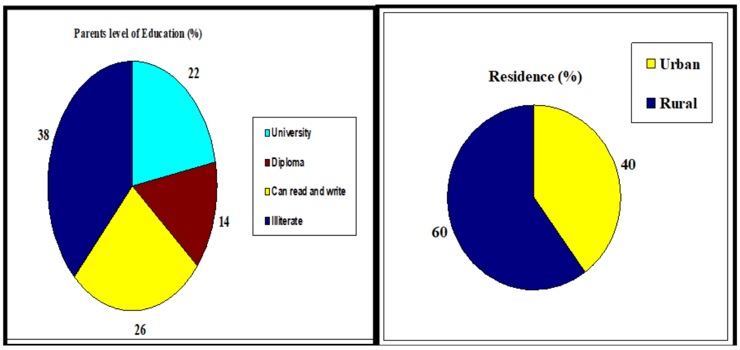

Figure (1): The distribution of the studied cases regarding parents level of education and residence.

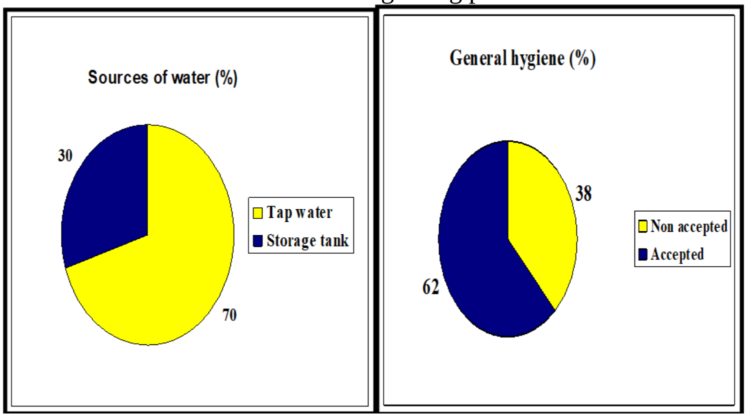

Figure (2): The distribution of the studied cases concerning the sources of water and general hygiene.

The intestinal parasitic infections with A. lumbricoides was positive in (14%) of the examined cases. Mixed infection with two parasites, Ascaris lumbricoides and Enterobius vermicularis, in the same patient formed (14%) of the infected cases, as shown in Table 2.

Table (2): Prevalence of A. lumbricoides infection among the examined cases.

Name of parasite	*n=300*			
	Positive		***Negative***	
	n	%	n	%
Ascaris lumbricoides	42	14%	258	86%
Mixed infection (*Ascaris lumbricoides with Enterobius vermicularis*)	42	14%	258	86%

A. lumbricoides infection was positive in 17.28% of the examined male samples and 10.17% of the examined female samples. Table 3 showed that there was an insignificant correlation between the frequency of A. lumbricoides infection and sex (p=0.076).

Table (3): Relation between sex and the A. lumbricoides infection

Ascaris lumbricoides	***n=300***				***P value***
	Male (n= 162)		***Female (n= 138)***		
	n	%	n	%	
Positive	28	17.28%	14	10.14%	0.076
Negative	134	82.72%	124	89.86%	

With regard to the age, there was also an insignificant correlation between the A. lumbricoides infection and age (p=0.363), as shown in Table 4.

Table (4): Relation between age and the A. lumbricoides infection (No= 300).

Ascaris lumbricoides	**Age (years)**				**P-value**
	1-4 (n= 30)	**4 - 8 (n= 120)**	**8 -12 (n= 138)**	**12 – 15 (n= 12)**	
Positive	4 (13.33%)	22 (18.33%)	15(10.87%)	1(8.33%)	0.363
Negative	26 (86.67%)	98 (81.67%)	123 (89.13%)	11 (91.67%)	

Upon the examination of Ascaris lumbricoides present in the collected stool samples by SEM, the anterior end revealed broad cervical alae and three large lips. Each lip had a pair of sensory papillae, and a pair of distinct pits in its smooth apical part. Each subventral lip possessed a simple antero-lateral papilla and an amphid. The inner circle of labial papillae was revealed as two distinct pits in the smooth apical part of each lip. A shallow pit was seen in the cuticle in the central region of the denticular row of all lips, as shown in Figure 3.

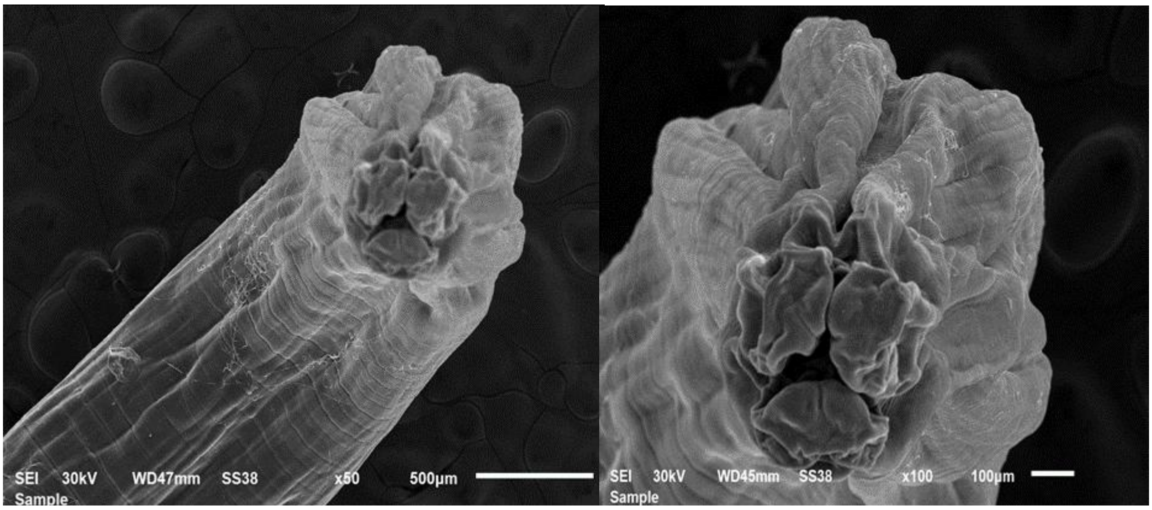

Figure (3): SEM images of A. lumbricoides (Type Egypt) anterior end, ventrolateral view.

A high-power SEM of portion of subventral lip showed an uninterrupted row of small, blunt, conoid denticles on inner labial surface, as shown in Figure 4.

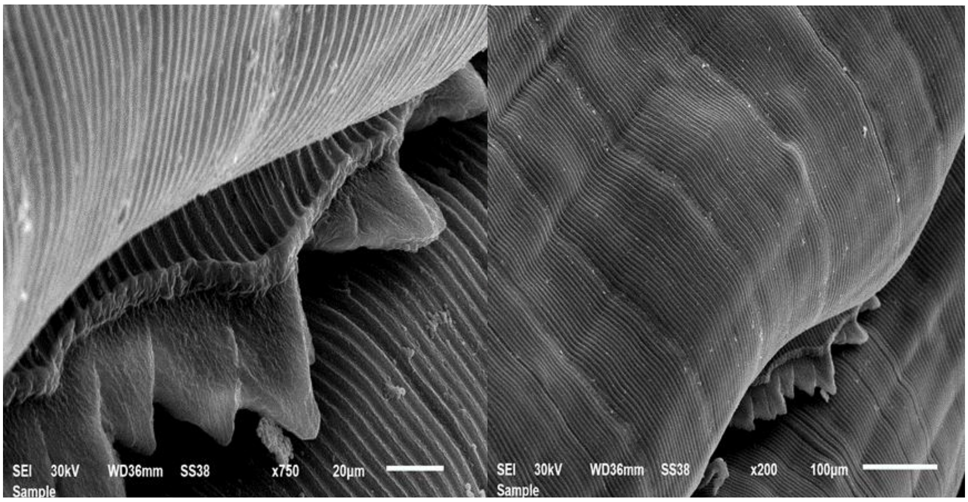

Figure (4): SEM images of the subventral lip of A. lumbricoides (Type Egypt) showed the denticles.

The cuticular striations were distinct along the entire length of the cervical alae, as shown in Figure 5. Additionally, the female A. lumbricoides had a straight blunt tail, or posterior end, while the male did not have any straight tail; rather, they were slightly coiled at the posterior side. In females, an anus was present just before the tail ended; this anus was guarded by lips. Only the digestive tube opened outside through the anus. In males, the anus was replaced by the cloaca, as shown in Figure 6.

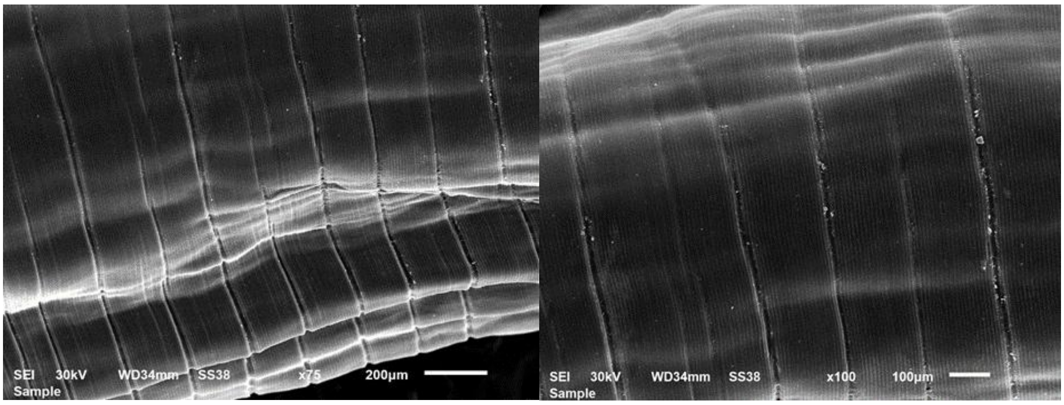

Figure (5): SEM images of cervical ala of A. lumbricoides (Type Egypt) showed the distinct cuticular striations.

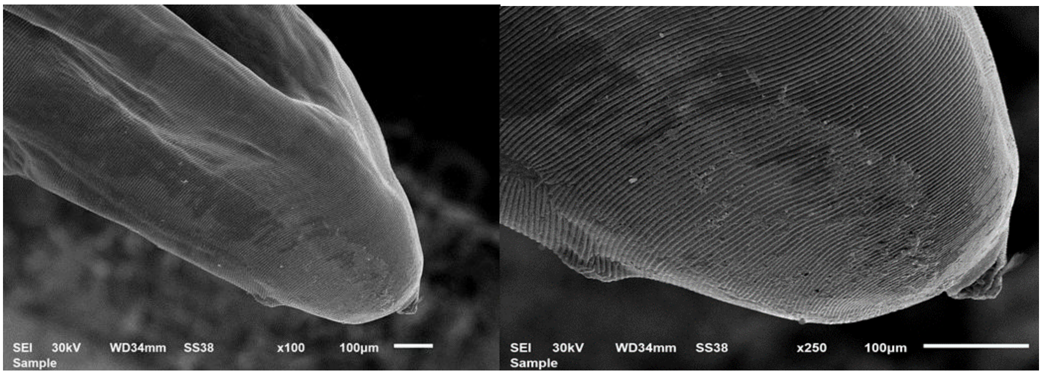

Figure (6): SEM images showed the posterior anal of adult female A. lumbricoides (Type Egypt).

4. DISCUSSION

The rates of intestinal nematode infections are high in children. These parasites are largely contagious, and poverty is a factor in their spread. As a result, locations with poor hygiene have a higher incidence of them (WHO, 2019).

Ascaris lumbricoides is one of the most common and largest nematodes identified in humans. It has a global distribution, infecting 25% of the world's population, and is particularly common in tropical and subtropical areas (Ali et al., 2020). The Ascaris lumbricoides worm is a member of the family Ascadidae, that belong to the order Ascaridida (phylum Nematoda). A. lumbricoides spreads through the intake of embryonated eggs contaminated with faeces. Risk factors include poverty, inadequate sanitation, improper sewage disposal, and poor personal hygiene. Children under the age of five have the highest prevalence (Leung et al., 2020).

The purpose of this research was to collect and analyze samples of children's faeces in order to study the prevalence of Ascaris lumbricoides (Type Egypt) as well as examining the ultrastructure using SEM.

Faecal samples were collected from the children involved in this study in clean plastic containers, and mothers were instructed to handle the samples soon after passing them. The samples were checked macroscopically for the presence of mucus, blood, or visible parasites. The Formalin ethyl-acetate concentration procedure was used to process the samples. A drop of sediment was placed on a microscope slide after concentration and covered with a cover-slip. The entire cover-slip was tested for the presence of eggs or worms using a 10x and 40x objective lenses.

Another Egyptian study performed by Monib et al (2016) included 260 children (158 males and 102 females), aged between four months and 15 years. 69 of the cases were infected with one or more parasites.

Allan et al (2020) revealed that the overall data was collected from 172 children aged 3 to 5 years in 5 villages within Kwa Njenga slum settlement in Nairobi County, Kenya. Of the 172 children sampled, 33.1% were 3 years old, 33.1% were 4 years old, and 33.7% were 5 years old. Among the 5 villages, 21.5% of children sampled were in Kwa Njenga, 19.8% in Vietnum, 19.2% in Wapewape, 19.8% in Kwa Reuben, and 19.8% in Motomoto.

Sitotaw and Shiferaw (2020) indicated that of the 383 study participants, the age ranged from 5 to 15 years, and most of the participants (83.3%) were 6-11 years old. In an Egyptian study by Ibrahium (2011), carried out on 264 randomly selected pupils, 93 were males and 171 females. Age ranged from 6 to 12 years, and more than three-quarters of the pupils (76.1%) were aged from 9 to 12 years.

In our study, we found that, in terms of sex, there was a male predominance; about 54% of cases were male and 46% were female. Another study by Sitotaw and Shiferaw (2020) showed that of the 383 study participants, female participants were slightly higher (51.2%) than males (48.8%).

We found that parents mainly were illiterate in 38% of cases followed by can read and write in 26%, university in 22% and diploma in 14%. Regarding residence, 60% were from rural areas and 40% were from urban areas. This is explained as, faster transmission of these parasites is facilitated by households lacking cemented floors, absence of health and hygiene education, deficiency of uncontaminated channelled water, ill-sustaining latrines, and children walking without shoes (Loukouri et al., 2019). Although urbanisation can stimulate access to health facilities and public works, congestion and poor hygiene will lead to higher contamination rates through the faster closeness of the infested to larger susceptible inhabitants (Angeles et al., 2009).

Sitotaw and Shiferaw (2020) also informed that of the 383 study participants, about half were urban dwellers; mostly from families with a farmer father (87%) and a housewife mother (91%), and most were from families who earned Birr 800-2,000 monthly. The majority of participants were mothers who attended only primary school (90.6%), and a little more than half (57.7%) were fathers who attended only primary school.

Moreover, most of the participants (91%) came from family sizes of 5 or more, and there was a statistically significant correlation between the intestinal parasitic infection and some other sociodemographic factors, including residence, family income, educational status, and occupation of the parents.

Moreover, a significantly higher prevalence rate of intestinal parasitic infections was noticed among students living in rural (76.4%) than urban (49.8%) areas. Similarly, students from families who earned less than Birr 800 (Ethiopian currency) were more infected (86.5%) compared with families who earned more than Birr 800 (44%). Likewise, students from families who attended only primary school were more infected (64.5-71.1%) compared with those from families who attended secondary school and above (41.7-50.6%). The occupation of mother and father also had a significant effect on the infection rate (Sitotaw and Shiferaw, 2020).

In contrast to Ibrahium, who showed that the majority of the pupils' parents were illiterate (72.7% of fathers and 81.8% of mothers), the majority of the pupils' parents were manual workers (80.3% of fathers and 90.9% of mothers) (Ibrahium, 2011).

We found that 70% of cases get their water from tap sources, 30% from storage tanks, and 62% of cases have accepted hygiene. In contrast, Sitotaw and Shiferaw (2020) informed that the prevalence of intestinal parasitic infection among the participants who had varying habits (either always, sometimes, or not all) of shoe wearing, hand washing after toilet use, and eating unwashed fruits and vegetables was statistically significant.

Similarly, the cleanliness of students' fingernails (clean or unclean), place of defecation (in latrine or open field), source of drinking water (river, pipe, or well), and ways of waste disposal (open dump, burn, or burry) had significant effects on the prevalence rate of intestinal parasitic infections among the participants.

The infections with A. lumbricoides was present in 14% of the studied cases. Likewise, mixed infection with two parasites, Ascaris lumbricoides and Enterobius vermicularis, in the same patient was present in 14% of the examined cases. There was no significant difference in the incidence of A. lumbricoides infection in terms of age and sex. In Ibrahium (2011) research, it was found that A. lumbricoides accounted for 3.8% of pupils; (3.2% of males and 4.1% of females). Allan et al (2020) reported that the distribution of A. lumbricoides did not markedly vary according to age.

Scanning electron microscopy (SEM) offers us with information on the sample's surface and its composition. This technique is based on detecting the reflected or knocked-off electrons to create an image when a beam of electrons is directed towards the sample. Upon SEM examination, the anterior end of A. lumbricoides revealed broad cervical alae and three large lips, one dorsal and two subventral, with the inner face of the free edge of each lip bearing an uninterrupted row of small, blunt, conoid denticles along almost its entire length. The denticles were almost of equal size and typically unicuspid.

Four big papillae—two ventrolateral on each subventral lip and one dorsolateral on the dorsal lip—represented the external ring of papillae. At higher magnification, such papillae looked to be single structures with a pore on their broad side that could be an amphid, separated from the cuticular surface by a distinct surface groove all around.

A straightforward antero-lateral papilla and an amphid were exist on each subventral lip. The smooth apical portion of each lip revealed two distinct pits that make up the inner circle of labial papillae. In the middle of each lip's denticular row, a small pit in the cuticle was visible. Along the whole length of the cervical alae, there were prominent cuticular striations.

Likewise, the description of A. lumbricoides (type Egypt) was similar to the micromorphological features identified by Ansel and Thibaut (1973) and Soulsby (1982).

5. CONCLUSION

Early identification of reported clinical manifestations may help in the early detection of A. lumbricoides infection and effective treatment. Further studies with a larger sample size and in different provinces are required to confirm the prevalence of this parasite in Egypt. Necessary sanitary strategies, health education, improvements in socio-economic conditions, screening, and deworming of intestinal parasites among children are recommended. Electron microscopy is essential for parasite ultrastructure-based drug discovery.

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