

FREQUENCY OF GASTROINTESTINAL PARASITES AMONG CHILDREN IN CLINICAL PRACTICES PESHAWAR, KPK, PAKISTAN

Muhammad Ilyas¹, Inayatullah Khan², Fazal ur Rehman³, Muhammad Shahid⁴

ABSTRACT

Objective: Chronic parasitic infections severely affect the cognitive development of young children. The current study was designed to determine frequency of gastro intestinal parasites among the children below 15 years of age.

Methods: A total of 700 stool samples from male (n=380) and female (n=320) children were collected during December 2016 and August 2017. The samples were examined for the presence of ova and cysts by routine direct microscopy and stool concentration technique (Formal ether sedimentation).

Results: The overall prevalence of *Giardia lamblia* (18.5%) followed by *Entamoeba histolytica* (7.8%), *Hymenolepis nana* (6.2%), *Ascaris lumbricoides* (1.4%) and *Ancylostoma duodenale* (0.85%) were recorded. Significance prevalence of 38.8% was recorded for Giardiasis in age 1-5 years. It was followed by 15.7% (6-10 years), 9.7% (11-15 years) and 8.2% (<01 year). High prevalence of *Entamoeba histolytica* (13.8%) was noted in the age group 1-5 years, followed by 7.3% (6-10 year), 5.5% (< 01years) and 4.3% (11-15 years). The prevalence of *Hymenolepis nana* was significant (12.2%) observed in the age group of 1-5 years. While *Ascaris lumbricoides* had significant prevalence of 2.2% within 1-5 years age group, which was followed by 1.6% (11-15 years) and 1.5% (6-10 years).

Conclusion: In the current study high prevalence of *Giardia lamblia*, *Entamoeba histolytica* and *Hymenolepis nana* observed in age group 1-5 years. Least infection was observed in <1 year age group. Co-infection between *Giardia lamblia* and *Hymenolepis nana* was also noted in 1-5 years age group. These infections may affect the physiological as well as psychological health in the growing children of the region, therefore, requiring proper remediation actions by concerned health quarters.

Keywords: *Giardia lamblia*, *Entamoeba histolytica*, *Ascaris lumbricoides*, Parasitic infections.

INTRODUCTION

Parasitic infestation affects the health of preschool and school aged children that may retard their mental and physical growth. Persistence parasitic infestation having long term consequences on their lives and pose significant hurdle on their learning and affecting their educational career. Chronic parasitic infections greatly influence to cognitive development of young children resulting in increased memory loss, diminished learning

ability and lowering overall intelligence quotient (IQ) levels. An average of 3.75 IQ points loss has been reported in children who were found chronically infected with gastrointestinal parasites¹. According to different epidemiological survey about 1.45 billion people were infected from soil transmitted helminths in which 5.19 million individuals showed morbidity in 2010. Out of 1.45 billion parasitic infections, 438.9 million people were infected by *Ancylostoma duodenale*, 819 million by *Ascaris lumbricoides* and 464.6 million by *Trichuris trichiura*¹¹⁻¹³. *Ascaris lumbricoides* is the most common and largest human intestinal parasite infected about one billion individual's worldwide¹⁴. Protozoanal infection also play pivotal role in contribution of intestinal diarrhea in children. *Giardia lamblia* (Protozoa) reported for high level of genetic diversity has global emergence and distribution. It has been estimated that about 200 million individuals have symptomatic giardiasis, and 500,000 new cases are reported each year especially in developing countries². Infection of the parasite occurs through fecal-oral route by direct or indirect contact with the infective stage of the parasite from anthroponotic, zoonotic and sapronotic sources^{3,4}. Human infections have been sourced to contaminated water, food, recreational water, house hold animals and infected individuals⁵. In developing countries it has been estimated that about 2.5 million deaths occurs per year and also affects

¹ Institute of Biological Sciences, Sarhad University of Science and Information Technology, Peshawar, KPK, Pakistan.

² Department of Pediatric unit Medical Teaching Institute Lady Reading Hospital Peshawar, KPK, Pakistan

³ Haematologist / Pathologist, M.Phil (Haematology), DCP (Pathology) City Medical Laboratory Peshawar, KPK, Pakistan

⁴ Department of Pharmacy, Sarhad University of Science and Information Technology Peshawar, Pakistan.

Address for correspondence:

Dr. Inayatullah Khan

Assistant Professor Pediatric unit Medical Teaching Institute Lady Reading Hospital Peshawar, KPK, Pakistan
Cell: 0345-9184168
Email: kinayat638@gmail.com

growth and cognitive function of the children^{6,7}. Amoebiasis the second most common endemic protozoan disease in developing countries as well as in developed countries. Travelers and immigrants play important role in its transmission^{8,9}. School going children are more commonly infected by *Hymenolepis nana* (cystode) called child worm and causing chronic diarrhea. It has been estimated that about 3.5 billion people were infected by this intestinal parasite worldwide¹⁰. Prevalence of this parasite was increasing globally particularly among the children¹⁵. Keeping in view the global prevalence of parasitic infections and their impact on children under age of 15 years, Present study was designed to estimate presence of gastrointestinal parasites among children of Peshawar city, Pakistan. The present findings will add to the existing knowledge about human intestinal parasite and more importantly to our best clinical practice.

METHODS

This study was conducted at City Medical laboratory Peshawar, one of the state of art diagnostic laboratory providing excellent quality diagnostic services in this region. More than five hundred patients are visiting daily the laboratory for their investigations. The current study was based on clinical practices in this region. Children under age fifteen years sent by local physicians (Children specialists) for stool R/E were included during December 2016 to August 2017. Demographic data like age and sex etc were collected on separate consent form.

Parents and guardians of children who brought the samples were informed on the purpose of study, possible outcomes, and significance to community. Samples were processed after their written informed consent.

Fecal sample collection and processing

A total of 700 stool samples from males (n=380) and females (n=320) children under fifteen years of age were collected. After proper instructions given to the children or guardian a single fresh fecal sample about 2 gram was collected in a leak proof and labeled container from each consenting study subject.

Preparation of smear in saline and Lugol's iodine

A small portion of stool was mixed with a drop of saline (0.85% sodium chloride solution) on clean glass slide and emulsified with wooden applicator. A clean glass cover slip was placed over the samples. It was examined for the presence of ova and cyst under 10× and 40× magnification of light microscope. Lugol's iodine staining preparation was also done to observe nuclei of the cysts.

Stool concentration technique

One gram stool sample was mixed with 10 ml of 0.85% saline solution in a 15 ml conical centrifuge tube, and centrifuged at 1500 RPM for one minute. After centrifugation, the supernatant was discarded and re-suspended the sediment with another 10 ml 0.85% saline solution. The process was repeated thrice to obtain clear sediment. The sediment was mixed with 7 ml of 10% formalin solution and mixed thoroughly by shaking the tube for 5 minutes. To this 3 ml of diethyl ether was added, stoppered the tube well and shake vigorously. After centrifugation at 2000 RPM for three minutes, supernatant was discarded and a drop of sediment was transferred to a clean glass slide and covered with cover slip. The entire area was examined for the presence of ova, cyst and larva of different intestinal parasites under 10× and 40 Magnification¹⁶.

Statistical analysis

Statistical analysis was performed by using GraphPad Prism 5 (GraphPad Software Inc. San Diego CA, USA). The Chi-square test (χ^2) was used to analyze the possible association between infection and exposure to different factors. The probability values were considered to be statistically significant when the calculated *P* value was equal to or less than 0.05.

RESULTS

In the current the overall prevalence of gastrointestinal parasites was 34.7%. In male it was 37.3% while 32.1% was reported in female. Among the total samples prevalence of *Giardia lamblia* was 130(18.5%) (*P* = 0.7805, OR = 1.056, 95% CI = 0.7199-1.549), followed by *Entamoeba histolytica* 55(7.8%) (*P* = 0.2427, OR = 1.399, 95% CI = 0.7946-2.463), *Hymenolepis nana* 44(6.2%) (*P* = 0.9715, OR = 1.011, 95% CI = 0.5477-1.867), *Ascaris lumbricoides* 10 (1.4%) (*P* = 0.3150, OR = 1.983, 95% CI = 0.5084-7.735) and *Ancylostoma duodenale* 06 (0.85%) (*P* = 0.1514, OR = 4.253, 95% CI = 0.4941-36.61).

Gender wise prevalence (Table 1) of *Giardia lamblia* was 72(18.9%) were found in males and 58(18.1%) in females. In males prevalence of other parasites, *Entamoeba histolytica*, *Hymenolepis nana*, *Ascaris lumbricoides* and *Ancylostoma duodenale* was 34(8.94%), 24(6.3%), 7(1.84%) and 5(1.31%) respectively, Whereas in females the prevalence of these parasites was 21(6.56%), 20(6.25%), 3(0.93%) and 1(0.31%) respectively.

Parasitic infection in various age groups showed in (Table 2). The overall prevalence observed under one year of age was 14.4%, followed by 71% (1- 5years), 34.2% (6-10 years) and 19.4% (11-15 years). Significant prevalence of *Giardia lamblia* was 38.8% (1-5 years), 15.7% (6-10 years), 9.7% (11-15 years) and 8.2% (<01 year) (*P* < 0.0001). High prevalence of *Entamoeba histolytica* (13.8%) (*P* < 0.0039) was noted in the age group of 1-5 years, followed by 7.3% (6-10 year), 5.5%

Table 1: Overall Prevalence of Gastrointestinal Parasites

| Parasites detected | Gender | | χ^2 | P value | Odds ratio | Confidence interval | Overall prevalence (%) |
|--------------------|-----------------------|-------------------------|----------|---------|------------|---------------------|------------------------|
| | Male (n = 380) No (%) | Female (n = 320) No (%) | | | | | |
| G. lamblia | 72 (18.9) | 58 (18.1) | 0.077 | 0.7805 | 1.056 | 0.7199-1.549 | 18.5 |
| E. histolytica | 34 (8.94) | 21 (6.56) | 1.365 | 0.2427 | 1.399 | 0.7946-2.463 | 7.8 |
| H. nana | 24 (6.3) | 20 (6.25) | 0.001 | 0.9715 | 1.011 | 0.5477-1.867 | 6.2 |
| A. lumbricoides | 7 (1.84) | 3 (0.93) | 1.009 | 0.3150 | 1.983 | 0.5084-7.735 | 1.4 |
| A. A. duodenale | 5 (1.31) | 1 (0.31) | 2.058 | 0.1514 | 4.253 | 0.4941-36.61 | 0.85 |
| Total | 142(37.3) | 103(32.1) | | | | | 34.7 |

Chi-square test was used and $P < 0.05$ was considered as significant at 95% confidence interval

Table 2: Age Wise Prevalence of Gastrointestinal Parasites

| Parasites detected | Age (years) | | | | χ^2 | P value |
|----------------------|-----------------------|----------------------|-----------------------|------------------------|----------|-------------|
| | < 01 (n = 145) No (%) | 1-5 (n = 180) No (%) | 6-10 (n = 190) No (%) | 11-15 (n = 185) No (%) | | |
| G. lamblia | 12 (8.2) | 70 (38.8) | 30 (15.7) | 18 (9.7) | 69.83 | < 0.0001*** |
| E. histolytica | 08 (5.5) | 25 (13.8) | 14 (7.3) | 08 (4.3) | 13.39 | 0.0039** |
| H. nana | 00 (00) | 22 (12.2) | 15 (7.3) | 07 (3.7) | 23.30 | < 0.0001*** |
| A. lumbricoides | 00 (00) | 04 (2.2) | 03 (1.5) | 03 (1.6) | 2.986 | 0.3938 |
| A. duodenale | 01 (0.6) | 02 (1.11) | 03 (1.5) | 00 (00) | 2.949 | 0.3996 |
| G. lamblia + H. nana | 00 (00) | 05 (2.77) | 00 (00) | 00 (00) | 14.55 | 0.0022** |
| Total | 21(14.4) | 128(71.1) | 65(34.2) | 36(19.4) | | |

Chi-square test was used and $P < 0.05$ was considered as significant

Table 3: Prevalence Reports of Gastrointestinal Parasites in Pakistan

| Parasite | Prevalence | References |
|-----------------|------------|------------|
| E. histolytica | 2.5 | 20 |
| G. lamblia | 19.8 | |
| A. lumbricoides | 22.8 | |
| H. nana | 4.6 | |
| G. lamblia | 3.09 | 21 |
| G. lamblia | 11.8 | |
| E. histolytica | 5.9 | |
| H. nana | 1.7 | |
| A. lumbricoides | 3.8 | 19 |
| E. histolytica | 46.5 | |
| G. lamblia | 16.0 | |

(< 01years) and 4.3% (11-15 years). Similarly, *Hymenolepis nana* had significant prevalence ($P < 0.0001$) in the age group of 1-5 years (12.2%), whereas in the age groups of 6-10 years and 11-15 years, the prevalence was 7.3% and 3.7% respectively. High prevalence of *Ascaris lumbricoides* 2.2% was observed in the age group of 1-5 years, followed by 1.6% (11-15 years) and 1.5% (6-10 years). The most susceptible age group to *Ancylostoma duodenale* was 6-10 years, where 1.5% prevalence was noted for this parasite. However, the prevalence of *Ascaris lumbricoides* and *Ancylostoma duodenale* infections in the different age groups was statistically insignificant.

In the present study mixed parasitic infection of *Giardia lamblia* and *Hymenolepis nana* were observed in only five cases (0.7%) in the age group 1-5 years (Table 2).

DISCUSSION

In developing countries like Pakistan, intestinal parasitic infestation is mainly an ailment of children due to poor personal hygiene. Adults, however, acquire the illness due to social and socioeconomic reasons compounded by the lack of elementary education about common human parasitic diseases. Unfortunately in this region there is no proper surveillance program for routine clinical examination among the communities. Parasitic infestation based on some environmental, biological, behavioral, socioeconomic and health systems factors. These infections are quite prevalent among the poor part of the population and contributed by factors like low household income, poor environmental sanitation, personal hygiene, overcrowding, limited access to clean water and tropical climate¹⁷. The present study revealed that the most common intestinal parasites were *Giardia lamblia*, *Entamoeba histolytica*, *Hymenolepis nana*, *Ascaris lumbricoides* and *Ancylostoma duodenale* (Hook worm).

Previous reports about prevalence of gastrointestinal parasites in other regions of Pakistan are shown in (Table 3). A high prevalence of *Entamoeba histolytica* (46.5% and 5.9%) was observed in rural area of Karachi and District Muzafarabad¹⁸⁻¹⁹. High prevalence of *Giardia lamblia* (19.8%, 16.0% and 3.0%) was also reported¹⁹⁻²¹. Prevalence of *Ascaris lumbricoides* and *Hymenolepis nana* were 22.8% and 4.6%²⁰, while in the present study, prevalence of *Giardia lamblia* was (38.8%) in age group 1-5 years, which is quite similar to the findings of Mehraj and his coworkers¹⁷. Age factor is an important segment regarding the contribution of parasitic infection in pre-school and school going children. In the current study Giardiasis and Amoebiasis were the frequently encountered infections in all age groups. Another common intestinal parasite was *Hymenolepis nana* (child worm), which was found in all age groups except under age 1 year. Our findings are in conformity with earlier reports¹⁷⁻²¹.

In children, chronic parasitic infection particularly *Ancylostoma duodenale* (Hook worm) infestation retards physical growth, which is sometimes most apparent at puberty²². More recent evidence suggests that parasitic infection also has subtle yet profound adverse effects on memory, reasoning ability and reading comprehension in childhood. Most of these effects are probably attributable to the presence of iron-deficiency anemia. Infants and pre-school children are particularly vulnerable to the developmental and behavioral deficits caused by iron-deficiency anemia²³⁻²⁴. Poverty, low socioeconomic status, contaminated water supply and unhygienic environment are the major factors, facilitating the incidence of parasitic infections especially in the developing countries. In the present study prevalence of *Ascaris lumbricoides* was (1.4%) which was lower (15%) than the previous report²⁵. This difference may be due to regional differences. Moreover defecation

in open fields and contaminated water supply are the factors increasing the risk of the parasitic infections among different population. In the current study the stool samples were collected from the children residing of district Peshawar city, where sewage pipes are in close proximity to drinking water supply pipes which may increase possibility of contamination of drinking water due to leakage of sewage pipes.

In the present study co-infection (*Giardia lamblia* and *Hymenolepis nana*) was (0.7%) observed in age group 1-5 years. However, an earlier report²⁰, suggests prevalence of co-infection (*Ascaris lumbricoides* + *Giardia lamblia*) and (*Ascaris lumbricoides* + *Trichurus trichiura*) was (0.84%) and (1.68%), respectively. This slight variation in co-infection among the parasites might be due to differences in geographical and climate distribution among the investigated regions, variation in drinking water sources and eating habits among the population.

A study conducted in south region of KPK²¹ reported, *Taenia saginata* was the most frequently parasite in children under age 12 year, which reflected an excessive intake of meat and its products in that particular region. Moreover consumption of infected livestock by the population may be the reason in that report. However, in present study we did not observe a single case infected from this parasite, but on other hand a high prevalence of Giardiasis and Amoebiasis was observed that may be due to contaminated water and commonly sold unhygienic street food in this region. To eradicate parasitic infestations control measures like water filtration, avoiding partially cooked meat and its products, treatment of infected livestock and screening for parasites in symptomatic individuals is required. Moreover public awareness is needed especially for the impact of these parasites on children physical and mental health.

CONCLUSION

A high prevalence of *Giardia lamblia*, *Entamoeba histolytica* and *Hymenolepis nana* is observed among the children under age fifteen years. The age group 1-5 year was found to be more affected. These parasitic infections may have physiological as well as psychological implications in young children that need proper remedial strategies and actions by the concerned public health quarters as well as the non government organizations.

REFERENCES

1. World Health Organization, 2003. Washington DC.
2. World Health Organization, 1996. Geneva.
3. Caccio SM, Thompson RC, Mclauchlin J, Smith HV. Unravelling Cryptosporidium and Giardia epidemiology. Trends Parasitology 2005; 21: 430-437.
4. Hubalek Z. Emerging human infectious diseases,

- Anthroponoses, zoonoses, and sapronoses. *Emer Infects* 2003; 9: 403–404.
5. Dillingham RA, Lima AA, Guerrant RL. Cryptosporidiosis. Epidemiology and impact. *Microb Infect* 2002; 4:1059–1066.
 6. Kosek, Bern MC, Guerrant RL. The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. *Bull WHO* 2003; 81: 197–204.
 7. Murray CJL, Lopez AD. Alternative projections of mortality and disability by cause 1990–2020: Global burden of disease study. *Lancet* 1997; 349: 1498–1504.
 8. Gathiram V, Jackson TFHG. A longitudinal study of asymptomatic carriers of pathogenic zymodemes of *E. histolytica*. *S. Afr. J. Med. Sci* 1987; 72: 669–672.
 9. Haque R, Ali IKM, Petri WA. Prevalence and immune response to *Entamoeba histolytica* infection in pre-school children in Bangladesh. *Am. J. Trop. Med* 1999; 60: 1031–1034.
 10. Brooker S, Kabatereine NB, Smith JL, Mupfasoni D, Mwanje M. An updated atlas of human helminth infections: the example of East Africa. *Int J Health Geogr* 2009; 8: 42.
 11. Hotez PJ, Alvarado M, Basanez MG, Bolliger I, Bourne R. The Global Burden of Disease Study 2010: Interpretation and Implications for the Neglected Tropical Diseases. *PLoS Negl Trop* 2014; 8(7): e2865.
 12. Pullan RL, Smith JL, Jasrasaria R, Brooker SJ. Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. *Parasites & Vectors* 2014; 7:37.
 13. World Health Organization. Soil-transmitted helminthiasis. Number of children treated 2007–2008: update on the 2010 global target. *Wkly Epidemiol Rec* 2010; 85: 141–8.
 14. Pillai DR, Kain KC. Common Intestinal Parasites. Current Treatment Options in Infectious Diseases 2003; 5: 207–217.
 15. Lindo FJ, Levy AV, Baum KM, Palmer JC. Epidemiology of giardiasis and cryptosporidiosis in Jamaica. *Am J Trop Med Hyg* 1998; 59(5): 717–721.8.
 16. Gelaw A, Nigussie BAB, Silesh B, Yirga BA, Alem M, Endris M, Gelaw B. Prevalence of intestinal parasitic infections and risk factors among schoolchildren at the University of Gondar Community School, Northwest Ethiopia: a cross-sectional study. *BMC Public Health* 2013; 13: 304.
 17. Mehraj V, Hatcher J, Akhtar S, Rafique G, Beg MA. Prevalence and Factors Associated with Intestinal Parasitic Infection among Children in an Urban Slum of Karachi. *PLoS ONE* 2008; 3(11): e 3680.
 18. Chaudhry ZH, Afzal M, Malik MA. Epidemiological Factors Affecting Prevalence of Intestinal Parasites in Children of Muzaffarabad District. *Pakistan J. Zool* 2004; 36 (4): 267-271.
 19. Siddiqui MI, Bilqees FM, Iliyas M, Perveen S. Prevalence of Parasitic Infections in a rural area of Karachi, Pakistan. *JPMa* 2002; 52: 315.
 20. Ahmed K, Shezana, Jan M, Imran R, Shuja N. Prevalence of Intestinal Parasitic Pathogens Among Gastroenteritis Patients in District Gilgit, Gilgit-Baltistan, Pakistan Shah G. *Pakistan J. Zool* 2012; 44(4): 1059-1063.
 21. Mirza IA, Kazmi SY, Yasir M. An analysis of intestinal parasitic infestation in dera Ismail khan, Pakistan. *J Ayub Med Coll Abbottabad* 2012; 24(1):123-124.
 22. Stephenson LS, Latham MC, Kurz KM, Kinoti SN, Brigham H. Treatment with a single dose of albendazole improves growth of Kenyan schoolchildren with hookworm, *Trichuris trichiura*, and *Ascaris lumbricoides* infections. *Am J Trop Med Hyg* 1989; 41: 78- 87.
 23. Lozoff B, Jimenez E, Hagen J, Mollen E, Wolf AW. Poorer behavioral and developmental outcome more than 10 years after treatment for iron deficiency in infancy. *Pediatrics* 2000; 105:E51.
 24. Sakti H, Nokes C, Hertanto WS. Evidence for an association between hookworm infection and cognitive function in Indonesian school children. *Trop Med Int Health* 1999; 4: 322-34.
 25. Shoaib MK, Jehan S, Akram M, Rabnawaz, Zaib M, Latif Z, Hussian F, Naeem M. Prevalence of Intestinal Protozoan & Worms Infestation in Primary School going Children Of 5-10 years of age, in District Bannu. *Ann. Pak. Inst. Med. Sci* 2012; 8(4): 243-248.