Research Article

The Epidemiology of Enterobiasis and Molecular Characterization of *Enterobius vermicularis* in Children of Karachi, Sindh, Pakistan

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Abstract | Pinworms (*Enterobius vermicularis*) belong to the Oxyuridae family which cause enterobiasis, under unsanitary conditions, the Oxyruidae family causes health concerns, particularly in children. Samples were collected from the laboratory of Jinnah Hospital, Karachi, Sindh, Pakistan. The data of 60 pinworm-infected children were recorded. These children were of various sexes and ages. Pinworm prevalence in children by gender from 2019 to 2020 demonstrates that females have a higher rate of incidence than males because of unsanitary circumstances and hormone production. Traditional pinworm diagnosis via PCR for pinworm detection has been performed. The findings in this research were based on two key factors: molecular analysis and statistical analysis. When traditional morphological techniques used with, molecular biology technologies it has proven to be effective in distinguishing closely related species. Statistical analysis is used to determine the intensity of infection in relation to age and gender. This study will focus on children who are suspected of being afflicted with pinworms. The presence of adult worms or ova in the feces is required to make proper diagnosis. The swabbing of the perianal region is utilized to determine the presence of eggs in feces.

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Introduction

Pinworms (*Enterobius vermicularis*) of the family Oxyuridae cause oxyuriasis/ enteterobiasis. The worm has been known since antiquity and Linnaeus was the first to describe it in 1758. Pinworms are common in birds, mammals, reptiles and amphibians but they are rare in fish and absent in dogs and cats, which can take up pinworm eggs from the environment and infect humans through their fur. This microscopic nematode is found all across the world especially in children. It is the most common parasitic infection in the world. Its entire life cycle was also described in detail (Leuckart, 1865).

E. vermicularis is one of the most common parasitic helminth in humans. It was estimated that over 209 million people are sick around the world, with

children aged 5 to 10 making up more than a 30% of those affected (Cook, 1994; Kucik *et al*., 2004; Fan *et al*., 2019; Rawla and Sharma, 2021). Despite the fact that enterobiasis is unrelated to socioeconomic class, race, or culture, it is facilitated by factors such as poor personal or group hygiene as well as overcrowding (in preschools, schools, orphanages, and family groups) (Cook, 1994; St Georgiev, 2001; Burkhart and Burkhart, 2005). Pinworm eggs are transmitted from person to person in these conditions, either directly through the anus-to-mouth pathway, by finger contamination, or indirectly through contaminated objects (toys, food) (Cook, 1994; Vermund and Wilson, 2000). Majority of *E. vermicularis* infections are asymptomatic or produce ambiguous symptoms such as perianal pruritus, which leads to local epidermal irritation and bacterial infections. Other symptoms include abdominal discomfort, loss of appetite, weight loss, sleepiness, restlessness and irritability (Burkhart and Burkhart, 2005; Vermund and Wilson, 2000). Pinworm infection can cause enuresis (Çulha and Duran, 2006; Otu-Bassey *et al*., 2011) however it is rare. In the present study, the research findings were based on two primary parameters, molecular and statistical analyses, to be specific. Statistical analysis of obtained data based on age, gender, pinworm count, and infection severity. According to current research on pinworm, it is the most common condition in girls as compared to boys. Molecular research revealed that the specimens recovered during this study were strikingly comparable to previously collected *E. vermicularis* isolates from around the world. The present study has determined the severity of infection in the general public in order to design safe prophylactic strategies to protect individuals against pinworm also demonstrates that cleanliness and personal hygiene are major protective factors.

Materials and Methods

Samples were collected from the laboratory of Jinnah Hospital, Karachi, Sindh, Pakistan. From the recorded data, 60 children were found positive for pinworm-infection. These children were of various sexes and ages. Traditional pinworm diagnosis, PCR for pinworm detection has been performed. The findings in this research are based on two key factors: Molecular analysis and statistical analysis. When used with traditional morphological techniques, molecular biology technologies have proven to be effective in distinguishing closely related species according to the method of Zelck *et al*. (2011).

Pinworms were found in toddler excrement that had been magnified, cleaned in tap water, and kept at 20°C. Then, to extract DNA, pinworms from 37 kids who lived in various parts of Germany were thawed and mechanically homogenised (Qiamp DNA minikit, Qiagen, Hilden, Germany). Using universal primers, the ribosomal DNA (5S rDNA) of *E. vermicularis* was amplified and sequenced as partly overlapping fragments of %S. Each primer was 100 nM, and each deoxynucleoside triphosphate (dNTP) was 50 M. The amplification reaction mixtures (50 µl) also contained 2.5 mM $MgCl_2$, 0.5 units of polymerase, and 10 µl of template. The following steps were used in the PCR amplification process: (i) denaturation at 95°C for 5 min; (ii) 40 cycles, with each cycle consisting of 1 cycle that lasted 60 s at 94°C, 60 s at 50 to 60°C, and 2 min at 72°C for 2 min; and (iii) a final extension step lasting 10 min at 72°C. Under otherwise comparable circumstances, 5S rDNA fragments were amplified for diagnostic purposes at 55°C using the Enterobius-specific primers Ev18S.F1 and Ev18S. R1. On agarose gels stained with ethidium bromide, products were found. PCR products were sequenced using a BigDye terminator cycle sequencing kit and an ABI Prism 310 genetic analyzer either directly or after gel extraction (QIAquick gel extraction kit; Qiagen, Hilden, Germany) and cloning (TOPO-TA; Invitrogen, Karlsruhe, Germany) (Applied Biosystems, Warrington, United Kingdom). When the heights of the current alternative nucleotide peaks were equal, or when a minor peak greatly outperformed the background level and made up 50% of the primary peak, site polymorphisms were assessed.

Statistical analysis is used to determine the intensity of infection in relation to age and gender. The core data, which included 60 children, was entered into a Microsoft Excel spreadsheet, for constructing graphs, whereas chi-square test calculated by using the statistical program Statistical Package for Social Students (SPSS version 18.0).

Results and Discussion

Molecular analysis

The total gDNA from individual pinworms (14 specimens) was separately extracted and the PCR amplification was performed using the extracted

gDNA of the pinworms with primers and PCR conditions as specified. By utilizing the Nanodrop ND-200 instrument to measure the gDNA content in *E. vermicularis* samples, 260/280 absorbance measurement peaks were calculated for the accuracy of gDNA concentrations, the amount of DNA was represented as ng/µl Figure 2. The first round of PCR yielded the 122 bp band on agarose gel for some specimens (EV1, EV2, EV3, EV7, EV8, EV10, EV11, EV12, EV13 and EV14) and also in the second round of PCR using the same primers and PCR outcomes of the first round of PCR as a template for DNA reaction yielded the 122 bp band for all specimens (Figure 3A and B).

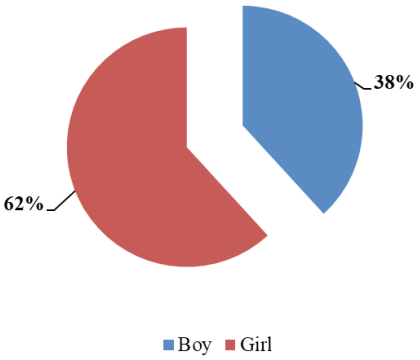

Figure 1: *Pie chart showing the prevalence of pinworms in children by gender during 2019 to 2020.*

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Cursor Pos.	Cursor abs.	340 raw
EV1	Default	1/4/1980	12:19 AM	120.67	2.413	1.433	1.68	2.04	50.00	230	1.181	0.120
EV2	Default	1/4/1980	12:20 AM	157.46	3.149	1.810	1.74	2.27	50.00	230	1.390	0.018
EV3	Default	1/4/1980	12:21 AM	113.42	2.268	1.502	1.51	2.02	50.00	230	1.123	0.045
EV4	Default	1/4/1980	12:23 AM	25.70	0.514	0.259	1.99	1.51	50.00	230	0.341	0.004
EV5	Default	1/4/1980	12:24 AM	25.58	0.512	0.249	2.05	1.75	50.00	230	0.291	0.018
EV6	Default	1/4/1980	12:26 AM	24.43	0.489	0.238	2.05	1.87	50.00	230	0.262	0.002
EV7	Default	1/4/1980	12:28 AM	69.06	1.381	0.816	1.69	0.85	50.00	230	1.627	0.192
EV8	Default	1/4/1980	12:29 AM	121.69	2.434	1.434	1.70	2.04	50.00	230	1.194	0.023
EV9	Default	1/4/1980	12:31 AM	125.02	2.500	1.494	1.67	2.04	50.00	230	1.228	0.023
EV10	Default	1/4/1980	12:32 AM	65.75	1.315	0.773	1.70	0.87	50.00	230	1.515	0.138
EV11	Default	1/4/1980	12:34 AM	154.71	3.094	1.773	1.74	2.29	50.00	230	1.352	0.047
EV12	Default	1/4/1980	12:35 AM	156.01	3.120	1.799	1.73	2.27	50.00	230	1.373	0.045
EV13	Default	1/4/1980	12:35 AM	225.66	4.513	2.478	1.82	2.19	50.00	230	2.058	0.077
EV14	Default	1/4/1980	12:37 AM	228.06	4.561	2.506	1.82	2.22	50.00	230	2.059	0.081

Figure 2: *The determination of gDNA concentration of Enterobius vermicularis specimens using Nanodrop ND-200 system. The accuracy of gDNA concentrations were calculated using the peaks of absorbance measurements at 260/280 and quantity of DNA was expressed as ng/µl. EV represents Enterobius vermicularis specimens.*

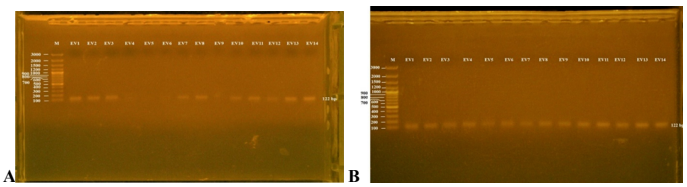

Figure 3: A: *The PCR amplification of target DNA fragment of 5S rRNA gene of E. vermicularis shows a 122 bp PCR product on agarose gel. M represents the marker of 100 bp Plus DNA ladder, starting from 100 with gradating band up to 3000 bp (Thermo Scientific).* **B:** *The second round of PCR amplification of target DNA fragment of 5S rRNA gene of E. vermicularis shows a 122 bp PCR product on agarose gel. M represents the marker of 100 bp Plus DNA ladder, starting from 100 with gradating band up to 3000 bp (Thermo Scientific). EV represents E. vermicularis specimens.*

The PCR products were purified as described and sequenced (4 specimens) by using 5S rRNA gene both forward and reverse primers for *E. vermicularis*. The results indicate that all the sequences aligned which belong to the same species with 100% similarity (Figure 4) whereas the sequences aligned was also estimated for *E. vermicularis* with other related species (Figure 5).

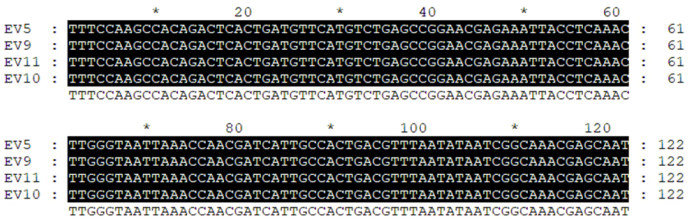

Figure 4: *Multiple alignment of sequenced specimens of E. vermicularis shows the 100% similarity indicating that all the specimens belong to same species.*

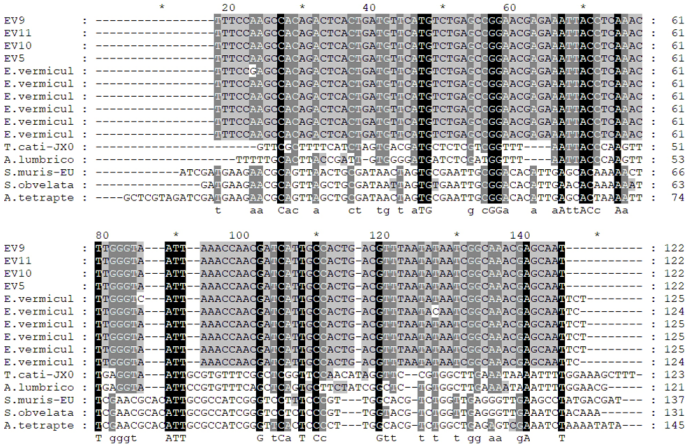

Figure 5: *Multiple alignment of sequenced specimens of E. vermicularis with other related species.*

The 5S rRNA gene fragment of *E. vermicularis* was amplified and sequenced and the DNA sequences obtained were compared for similarity check. In the MEGA 6.0 programme, the phylogenetic tree was built using nucleotide sequences from various helminths, with partial deletion for gaps and missing data, Poisson correction for nucleotide substitutions, and uniform rates among sites, were bootstrapped 10,000 times with bootstrap values of branches shown in percentages. Results indicate that the specimens collected during the present study were highly similar with already studied isolates from different regions of the world. Furthermore, the phylogenetic tree analysis shows that the *E. vermicularis* falls in a separate clade from other pinworms (*Syphacia obvelata*, *Syphacia muris* and *Aspiculuris tetraptera*) indicating that the *E. vermicularis* is genetically distinct from other pinworms (Figure 6).

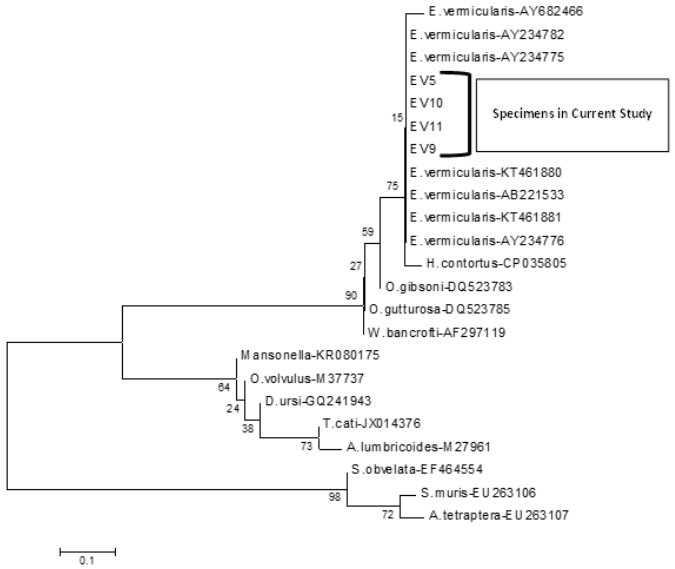

Figure 6: *Evolutionary analysis of pinworms (E. vermicularis) collected during the present study and compared with other related helminths using the phylogenetic tree constructed with neighbor-joining method.*

Statistical analysis

Statistical analysis of obtained data based on age, gender, pinworm count, and infection severity. According to present research on pinworm, it is the most common condition amongst girls as compared to boys during the years 2019 to 2020, with the help of the frequency distribution of children by gender. The prevalence was estimated using percentages. Table 1 and Figure 1 are showing mean intensity of pinworms in children by gender. Figures depict that, the mean intensity in boys than in girls. However, the association between gender and number of pinworms was calculated by chi-square test of value 12.87. Since, the $p<0.05$ which is statistically significant showing that there was an association between gender and number of pinworms.

Table 1: *Prevalence of pinworms in children by Gender During 2019 to 2020.*

Gender	Pinworms	Infected	Mean intensity	Chi-square	P value
Boy	43	21	2.04	12.87	0.045
Girl	129	39	3.30		

E. vermicularis commonly known as pinworm (Ridley, 2012), has a worldwide distribution and is one of the most prevalent helminth infections in children in the developed world (Cook and Zumla, 2008). Over one billion people are infected worldwide with a significant prevalence among children aged 5 to 6. Adults were less likely to be affected, implying that resistance develops with age, may be due to acquired immunity. Depending on age and race, infection rates increased up to 40% (Bowman and Lynn, 2009). Infection occurs when eggs were consumed or inhaled.

The small-subunit ribosomal DNA sequences were used to construct the molecular phylogeny of Nematoda where *E. vermicularis* had been included Nadler *et al.* (2007). Thus, presently 5S rDNA has been analyzed and compared for sequence together with other nematode sequences used in previous studies. The prevalence of enterobiasis is thought to be lower in the tropics (WHO, 1981), although this finding appears to be based on weak survey methodology (Haswell-Elkins *et al.*, 1987). The prevalence of enterobiasis has shown to diminish with increasing age in various investigations (Hayashi *et al.*, 1959; Rahman, 1991), implying that it is mostly a childhood infection. However, in surveys conducted in India, the illness was found to be prevalent across all age categories (Haswell-Elkins *et al.*, 1987). School students in a shantytown in Lima, Peru, had a prevalence of 42 percent (Gilman *et al.*, 1991), which was equivalent to the 40 percent rate recorded in a similar population in Indonesia (Norhayati *et al.*, 1994). Enterobiasis is much less common in blacks than it is in whites; the explanation for this disparity is unknown (Cram, 1943; Cherubin and Shookhoff, 1963). The incidence of *E. vermicularis* was 55 percent in an orthopaedic unit of a children's hospital in Liverpool, England (Ashford *et al.*, 1988). Extrapolating these figures to the entire country, at least 4.5 million children in the United States are infected with enterobiasis (Wagner and Eby, 1983). It was reported that, in tropical countries the percentage of incidence in children is surprisingly low as compared with the abundance of the other helminths. In warm countries rural life, scant loose clothing, sunshine dry heat, bathing facilities and toilet habits may provide conditions unfavorable for the existence of ova and transmission of infection. In cold countries crowded association in school and homes, lack of bathing and wearing of soiled clothing favor the transmission of the disease (Cram, 1941).

Random amplified polymorphic DNA (RAPD)-based assays based on polymerase chain reaction (PCR) have been successfully employed for the rapid and simple detection of genomic differences among parasites. The use of PCR provides a complementary strategy for parasite identification, and the genetic

variability of various species can be examined using the RAPD technique, which generates an aenornic fingerprint. Although there is a lot of material in the literature regarding employing molecular approaches for nematode identification and phylogenic analysis, there are just a few studies on pinworms. Some of these research used DNA sequence analysis to uncover pinworm evolutionary links (Nakano *et al*., 2006; Okamoto *et al*., 2007). A high-fidelity PCR was utilized in another work to simplify a major section of the ribosomal gene complex of *S. obvelata*, *S. inuris*, *A. tetraptera*, and *Passalurus ambiguus* collected from laboratory rodents and rabbits (Feldman and Bowman, 2007).

Conclusions and Recommendations

The oxyurides produce health problems under unhygienic conditions especially in children. It is imperative that the general public is made aware of the hazards of oxyurides and also of the methods of developing and maintaining hygienic conditions. Medication is the main medical therapy for pinworm infections but keeping the house clean and practicing proper hygiene is also vital throughout therapy.

Novelty Statement

The specimens of present study indicated high similarity with already collected isolates of *E. vermicularis* from different regions of the world but phylogenetic tree analysis shows it is genetically distinct and falls in a separate clade form other pinworms (*Syphacia obvelata*, *Syphacia muris* and *Aspiculuris tetraptera*).

Author's Contribution

Syeda Batool Zehra: Collected the samples.
Abdullah G. Arijo: Did molecular analysis.
Aly Khan: Did statistical analysis.
Nasira Khatoon: Provided literature.
Samina Waheed: Prepared the manuscript.

Conflict of interest
The authors have declared no conflict of interest.

References

Ashford, R.W., Hart, C.A. and Williams, R.G., 1988. *Enterobius vermicularis* infection in a children's ward. J. Hosp. Infect., 12(3): 221-224. https://doi.org/10.1016/0195-6701(88)90010-2

Bowman, D.D. and Lynn, R., 2009. *Helminths*. Georgis' parasitology for veterinarians, Saunders, St. Louis, USA. pp. 115-239.

Burkhart, C.N. and Burkhart, C.G., 2005. Assessment of frequency, transmission, and genitourinary complications of enterobiasis (pinworms). Int. J. Dermatol., 44: 837-840. https://doi.org/10.1111/j.1365-4632.2004.02332.x

Cherubin, C.E. and Shookhoff, H.B., 1963. The prevalence of enterobiasis, with regard to population group, among 500 children in New York City. Am. J. Trop. Med. Hyg., 12: 69-72. https://doi.org/10.4269/ajtmh.1963.12.69

Cook, G.C., 1994. *Enterobius vermicularis* infection. Gut., 35(9): 1159-1162. https://doi.org/10.1136/gut.35.9.1159

Cook, G.C. and Zumla, A.I., 2008. *Manson's tropical diseases*. Saunders Elsevier Health Sciences, Edinburgh UK.

Cram, E.B., 1941. Studies on Oxyuriasis. IX. The familial nature of pinworm infestation. Med. Annals, 10(2): 39-48.

Cram, E.B., 1943. Studies on oxyuriasis XXVIII. Summary and conclusions. Am. J. Dis. Child., 65(1): 46-59. https://doi.org/10.1001/archpedi.1943.02010130055003

Çulha, G. and Duran, N., 2006. The relationship between *Enterobius vermicularis* infection and nocturnal enuresis. Eur. J. Gen. Med., 3(1): 16-20. https://doi.org/10.29333/ejgm/82355

Fan, C.K., Chuang, T.W., Huang, Y.C., Yin, A.W., Chou, C.M., Hsu, Y.T., Kios, R., Hsu, S.L., Wang, Y.T., Wu, M.S., Lin, J.W., Briand, K. and Tu, C.Y., 2019. *Enterobius vermicularis* infection: Prevalence and risk factors among preschool children in kindergarten in the capital area, Republic of the Marshall Islands. BMC Infect. Dis., 19(1): 536. https://doi.org/10.1186/s12879-019-4159-0

Feldman, S.H. and Bowman, S.G., 2007. Molecular phylogeny of the pinworms of mice, rats and rabbits, and its use to develop molecular beacon assays for the detection of pinworms in mice. Lab. Anim., 36(9): 43-50. https://doi.org/10.1038/laban1007-43

Gilman, R.H., Marquis, G.S. and Miranda, E., 1991. Prevalence and symptoms of *Enterobius*

vermicularis infections in a Peruvian shanty town. Trans. R. Soc. Trop. Med. Hyg., 85(6): 761-764. https://doi.org/10.1016/0035-9203(91)90448-8

Haswell-Elkins, M.R., Elkins, D.B., Manjula, K., Michael, E. and Anderson, R.M., 1987. The distribution and abundance of *Enterobius vermicularis* in a South Indian fishing community. Parasitology, 95(Pt 2): 339-354. https://doi.org/10.1017/S0031182000057784

Hayashi, S., Sato, K., Takada, A., Shirasaka, R., Fukui, M., Sasa, M., Sukigara, H. and Hiraki, K., 1959. Studies on the epidemiology of pinworm (*Enterobius vermicularis*) in Japan. Jpn. J. Exp. Med., 29: 213-250.

Kucik, C.J., Martin, G.L. and Sortor, B.V., 2004. Common intestinal parasites. Am. Fam. Phys., 69(5): 1161-1168.

Leuckart, R., 1865. Entwicklungsgeschichte der Nematoden. Arch. Des Vereins f. gemeinsch. Arbeiten zur Förderung der wissenschaftlichen Heilkunde, 2: 200.

Linnaeus, C., 1758. Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Editio decima, reformata (10th revised edition), *Laurentius Salvius: Holmiae.* 1: 1-824. https://doi.org/10.5962/bhl.title.542

Nadler, S.A., Carreno, R.A., Mejía-Madrid, H., Ullberg, J., Pagan, C., Houston, R. and Hugot, J-P., 2007. Molecular phylogeny of clade III nematodes reveals multiple origins of tissue parasitism. Parasitology, 134(Pt 10): 1421–1442. https://doi.org/10.1017/S0031182007002880.

Nakano, T., Okamoto, M., Ikeda, Y. and Hasegawa, H., 2006. Mitochondrial cytochrome c oxidase subunit 1 gene and nuclear rDNA regions of *Enterobius vermicularis* parasitic in captive chimpanzees with special reference to its relationship with pinworms in humans. Parasitol. Res., 100(1): 51-57. https://doi.org/10.1007/s00436-006-0238-4

Norhayati, M., Hayati, M.I., Oothuman, P., Azizi, O., Fatmah, M.S., Ismail, G. and Minudin, Y.M., 1994. *Enterobius vermicularis* infection among children aged 1–8 years in a rural area in Malaysia. Southeast Asian J. Trop. Med. Public Health, 25(3): 494-497.

Okamoto, M., Urushima, H., Iwasa, M. and Hasegawa, H., 2007. Phylogenetic relationships of rodent pinworms (genus Syphacia) in Japan inferred from mitochondrial CO_1 gene sequences. J. Vet. Med. Sci., 69(5): 545-547. https://doi.org/10.1292/jvms.69.545

Otu-Bassey, I.B., Useh, M.F. and Alaribe, A.A., 2011. The post-treatment effects of enterobiasis on the occurrence of enuresis among children in Calabar, Nigeria. Asian Pac. J. Trop. Med., 4(4): 315-319. https://doi.org/10.1016/S1995-7645(11)60093-X

Rahman, W.A. 1991. Prevalence of *Enterobius vermicularis* in man in Malaysia. Trans. R. Soc. Trop. Med. Hyg., 85: 249. https://doi.org/10.1016/0035-9203(91)90043-X

Rawla, P. and Sharma, S., 2021. *Enterobius vermicularis.* In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan. PMID: 30725659.

Ridley, J.W., 2012. *Parasitology for medical and clinical laboratory professionals*. Delmar, USA. pp. 1-336. ISBN-13: 978-1-4354-4816-2.

St Georgiev, V., 2001. Chemotherapy of enterobiasis (oxyuriasis). Exp. Opin. Pharmacother., 2(2): 267-275. https://doi.org/10.1517/14656566.2.2.267

Vermund, S.H. and Wilson, C.M., 2000. Pinworm (*Enterobius vermicularis*). Semin. Pediatr. Infect. Dis., 11(4): 252-256. https://doi.org/10.1053/spid.2000.9639

Wagner, E.D. and Eby, W.C., 1983. Pinworm prevalence in California elementary school children, and diagnostic methods. Am. J. Trop. Med. Hyg., 32(5): 998-1001. https://doi.org/10.4269/ajtmh.1983.32.998

WHO. 1981. Intestinal protozoan and helminthic infections. WHO Tech. Rep. Ser., 58: 666-671.

Zelck, U.E., Bialek, R. and Wieb, M. 2011. Molecular phylogenetic analysis of *Enterobius vermicularis* and development of an 18S ribosomal DNA-targeted diagnostic PCR. J. Clin. Microbiol., 49(4): 1602-1604. https://doi.org/10.1128/JCM.02454-10