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Prevalence and genotype distribution of *Enterobius vermicularis* among kindergarteners in Shiraz and Khorramabad cities, IranAtefeh Tavan¹, Fattaneh Mikaeili¹✉, Seyed Mahmoud Sadjjadi¹, Sara Bajelan¹, Hossein Mahmoudvand², Meysam Sharifdini³¹Department of Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran²Department of Laboratory Sciences, School of Allied Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran³Department of Medical Parasitology and Mycology, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran

ABSTRACT

Objective: To study the prevalence and genotype of *Enterobius* (*E.*) *vermicularis* from adhesive tape samples in the cities of Shiraz and Khorramabad, Iran.

Methods: A total of 1 000 adhesive tape samples from kindergartens in Shiraz (500 samples) and Khorramabad (500 samples) were collected and tested using a microscope to find *E. vermicularis* egg/s. A questionnaire was filled out for each sample. In order to characterize the genotype of *E. vermicularis*, the PCR-sequencing method of the mitochondrial cytochrome C oxidase subunit 1 (*cox1*) gene was used. Genomic DNA was extracted from the positive scotch tape samples of *E. vermicularis*. The *cox1* gene was amplified by the polymerase chain reaction and sequenced. The sequence data were aligned using the BioEdit software and compared with the published sequences in GenBank. Phylogenetic analysis was performed using the maximum likelihood method.

Results: The parasitological method showed that 15 out of the 500 samples from Shiraz (3.00%) and 12 out of the 500 samples from Khorramabad (2.40%) were infected with *E. vermicularis* eggs. BLAST analysis indicated that the sequenced isolates belonged to *E. vermicularis* genotype B while three different haplotypes were also identified.

Conclusions: This is the first study on genotyping *E. vermicularis* in the cities of Shiraz and Khorramabad. Considering the public health importance of the disease, further studies are necessary to characterize the genotype of *E. vermicularis* in human populations from other regions of Iran.

KEYWORDS: *Enterobius vermicularis*; Genotype; Cytochrome C oxidase subunit 1 gene; Shiraz; Khorramabad; Iran

1. Introduction

Enterobius (*E.*) *vermicularis* (pinworm) is the most common intestinal nematode in children that causes enterobiasis or oxyuriasis[1]. This parasite is a direct-transmitted helminth such that its mode of transmission is direct interpersonal contact[2]. Enterobiasis is often asymptomatic in most adults and children, and the most important symptom of the disease is perianal pruritus[3,4]. Although *E. vermicularis* resides in the lumen of large intestine, it can occasionally be detected in ectopic sites such as the appendix, kidney, uterus, urinary tract and female genital tract, eye/s and subcutaneous nodule[5–13]. The best diagnostic method of enterobiasis is scotch tape test or graham method[14]. *E. vermicularis* is a cosmopolitan distributed nematode and children are the most commonly infected host. Epidemiological studies have indicated that the prevalence of *E. vermicularis* in human has been estimated from 0.3% to 66.14% in different parts of Iran[15–22].

Molecular studies have revealed the genotypes A, B and C in

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E. vermicularis from humans and chimpanzees[23–27]. Despite the relatively high prevalence of enterobiasis in the world, molecular studies for the genotype characterization of *E. vermicularis* are limited. The study conducted by Nakano *et al.* for the genotype characterization of *E. vermicularis* isolated from humans and chimpanzees showed that the type A belonged to human samples while the types A, B and C were detected in chimpanzee samples[23]. However, Piperaki *et al.*, Ferrero *et al.* and Kubiak *et al.* reported only the type B in human samples[24,25,27]. In a study of Rahnamaye Hayati Hagh *et al.* on genotype characterization of *E. vermicularis* in Iran, type B of *E. vermicularis* in humans was the predominant genotype of this nematode in the northwest of Iran[26]. Considering lack of information regarding the genotypes of *E. vermicularis* in the cities of Shiraz and Khorramabad, the present study was aimed to characterize the genotypes of this nematode from adhesive tape samples in these regions using the PCR-sequencing method.

2. Material and methods

2.1. Study areas

Shiraz (29°37'N 52°32'E), one of the oldest cities of Iran, is located in the south of the country, in a green plain at the foot of the Zagros Mountains, 1 500 meters above sea level. Shiraz is the fifth-most-populous city of Iran and the capital of Fars Province (Old Persian as Pars). It has a moderate climate, and is overall classed as a hot semi-arid climate. Around 300 mm (12 inch) of rain falls each year in the city, almost entirely in the winter months (Figure 1).

Khorramabad (33°29'16''N 48°21'21''E) is the capital of Lorestan Province, western part of Iran, and is located in the Zagros Mountains. It has an extremely hot summer with very low humidity, but its winter is sufficiently wet (Figure 1).



Figure 1. Geographic location of studies areas in Iran (Fars and Lorestan Provinces).

2.2. Sample collection

A cross-sectional study was conducted from April to December 2017. The sample size is calculated using the following formula: $n = Z_{1-\alpha/2} p (1-p)/d^2$. Where $Z_{1-\alpha/2} = 1.96$ at type I error of 5%, where P = prevalence based on previous studies, d = absolute error or precision.

According to meta-analysis study, approximately 17% of kindergarten and primary school children are infected with *E. vermicularis* in Iran[16]. The sample size was calculated with the absolute error or precision of 0.05 and at type I error of 5% (1.96). Therefore, based on the formula $n = 1.96^2 \cdot 0.17 \cdot (1-0.17)/0.05^2$. This gave a sample size of 217. To reduce errors, the sample size increased to 500. A total of 1 500 samples from 500 kindergarten children in Shiraz as well as 1 500 samples from 500 kindergarten children in Khorramabad were collected and examined using scotch tape test[28]. The procedure of sample collection was explained to the parents. In brief, the samples should be collected in the early morning before washing the perianal area. The adhesive side of the transparent scotch tape was placed firmly on the skin of the perianal area several times and fixed on the labeled microscope slide. The samples were transferred to the laboratory for microscopic examination. The parents of children were given consent forms. The consent form had two parts, one part was information sheet describing the research and the role of the participants, and another part was certificate of consent attesting to the participant's consent. A structured questionnaire was filled out for each sample. In order to improve the chances of detection of *E. vermicularis* eggs, three adhesive tape samples were taken from each child on three separate days. The design of the study, including ethical aspects, was reviewed and approved by the ethics committee at the Shiraz University of Medical Sciences (code: IR.SUMS.REC.1396.S176).

2.3. Microscopy method

All the samples were tested by microscopic examination to find *E. vermicularis* eggs. The slides containing *E. vermicularis* eggs were stored at 4 °C for DNA extraction.

2.4. Molecular methods

2.4.1. DNA extraction and PCR-sequencing

The genomic DNA from positive scotch tape samples of *E. vermicularis* was extracted using Tissue Genomic DNA Extraction Mini kit, with Proteinase (Yekta Tajhiz Azma kit, Cat. No. FATGK001) according to the manufacturer's instructions with a minor modification. A piece of tape containing *E. vermicularis* eggs was excised from each slide, immersed in 200 μ L TG1 buffer and 20 μ L of proteinase K, and

finally incubated at 56 °C for 1 h and at 60 °C overnight.

The mitochondrial cytochrome C oxidase subunit 1 (*coxI*) gene was subjected to analysis using the PCR reaction. The forward (EVM1: 5'-TTTTTGGTCATCCTGAGGTTTATATTC-3) and reverse primers (EVM2: 5'-CCACATTATCCAAAATAGGATTAGCC-3) were used for amplification of the *coxI* gene[24]. The PCR reactions were performed in a final volume of 25 µL. Each reaction contained 12.5 µL of the PCR master mix (2x Master Mix RED (Ampliqon, Denmark), (1.25 U Taq DNA polymerase, 200 µM of dNTPs and 1.5 mM of MgCl₂), 0.5 µL of each primer (12.5 pmol), 5 µL of template DNA and 6.5 µL of double-distilled water. The temperature profile was one cycle of 94 °C for 5 minutes to denature the double stranded DNA, followed by 35 cycles of 94 °C for 1 minute (denaturation), 57 °C for 1 minute (annealing), 72 °C for 45 seconds (extension), and a final extension of 72 °C for 10 minutes. Double-distilled water (DDW) instead of template DNA was included in each set of the PCR reaction as negative control and DNA extracted from adult worm of *E. vermicularis* as positive control. The PCR products were separated by electrophoresis on a 1.5% agarose gel and stained with Gel Red (GelRed™ Nucleic Acid Gel Stain, 10 000× in Water, cat. No. 41003); afterwards, the bands were visualized using a UV transilluminator followed by digital photography.

2.4.2. Sequencing and genotype characterization

The PCR products were sequenced in two directions using the same forward and reverse primers used in the PCR. The sequence results were edited by the Geneious software (www.geneious.com) and the consensus sequences were compared with the GenBank reference sequences using BLAST system (http://www.ncbi.nlm.nih.gov/) for genotype characterization. A phylogenetic tree was constructed with sequences obtained in the present study along with the reference sequences deposited in GenBank and reference sequences of *Syphacia obvelata* as the outgroup using the maximum likelihood method in the MEGA 5.0 software[29]. Bootstrap analyses (using 1 000 replicates) were carried out to determine the robustness of the finding. The sequences reported in this paper were deposited in GenBank.

2.5. Statistical analysis

Statistical analysis was done using statistical package for the social sciences (SPSS) software (version 21.0). The Chi-squared test was performed to evaluate the associations between *E. vermicularis* infection and variables such as gender and age. A value less than 0.05 was considered statistically significant.

Table 1. Demographic characterizations, results of 3 successive sampling from each positive case observed in the first, second or third times and the accession numbers of isolates in Shiraz and Khorramabad.

Sample	Isolate	Sex	Age	Positive			No. of eggs in each field (10×)	Accession No.
				1st time	2nd time	3rd time		
Shiraz								
1	Oe26	Female	5	-	+	-	2	MH802600
2	Oe31	Female	4	-	+	-	1	-
3	OeF100	Female	6	+	+	-	≥100	MH802597
4	OeF101	Male	6	+	-	-	≥100	MH802606
5	OeF102	Male	6	+	+	-	≥100	MH802607
6	OeF103	Male	5	+	-	-	≥100	-
7	OeF104	Male	6	-	+	-	≥100	MH802608
8	OeF105	Male	5	+	-	-	50	MH802602
9	OeF106	Male	5	+	+	+	≥100	-
10	OeF107	Female	5	-	+	-	5	-
11	OeF108	Male	4	+	-	-	50	-
12	OeF109	Male	6	+	-	-	5	MH802610
13	OeF110	Male	6	+	+	+	≥100	MH802611
14	OeF111	Male	5	+	+	-	50	-
15	O107	Male	4	+	-	-	50	MH802599
Khorramabad								
1	Oe338	Male	6	+	+	-	≥100	MH802601
2	Oe98	Female	6	+	-	-	≥100	MH802595
3	OeB23	Female	5	-	+	+	≥100	MH802596
4	Oe313	Female	5	-	+	-	15-20	MH802598
5	Oe105	Male	4	+	-	-	25	MH802609
6	OeK19	Female	5	-	+	-	5	MH802603
7	Oe309	Male	4	+	-	-	≥100	-
8	OeK29	Female	6	+	-	-	2	-
9	OeA23	Female	5	+	-	-	≥100	MH802604
10	OeK45	Female	5	-	+	-	2	MH802605
11	OeA27	Male	6	+	+	-	≥100	-
12	OeK69	Male	6	+	-	-	50	-

3. Results

3.1. Prevalence results

Among the 500 kindergarteners in Shiraz, 262 (52.4%) were males and 238 (47.6%) were females with the mean age of (4.92±0.87) years old. 500 kindergarteners in Khorramabad consisted of 245 males (49.00%) and 255 females (51.00%) and the mean age was (5.12±0.78) years old. A total of 15 (3.00%) out of 500 scotch tape samples from Shiraz and 12 (2.40%) out of 500 scotch tape samples from Khorramabad were positive to *E. vermicularis* eggs by microscopy. This result was obtained from three successive samplings from each person, so that positive cases were observed in one, two or three times, successively or alternatively (Table 1).

The kindergarteners infected with *E. vermicularis* eggs consisted of 16 males (16/27, 59.26%) and 11 females (11/27, 40.74%)

with the mean age of (5.2±0.75) years old. The infection rate of the kindergarteners with *E. vermicularis* eggs in Shiraz and Khorramabad for males and females was 3.1% (16/507) and 2.2% (11/493), respectively. Although higher levels of infection rate with *E. vermicularis* were reported among boys than in girls, there was no statistically significant association between gender and infection rate ($P=0.367$). Based on the statistical analysis, the difference between the age of the kindergarteners and infection rate with *E. vermicularis* was not significant ($P=0.197$).

3.2. Genotyping results

The positive scotch tape samples of *E. vermicularis* were examined by the PCR method using the mitochondrial *cox1* gene. An approximately 390 bp band was amplified from 27 isolates and the positive control after the PCR, and no amplification was observed in the negative controls.

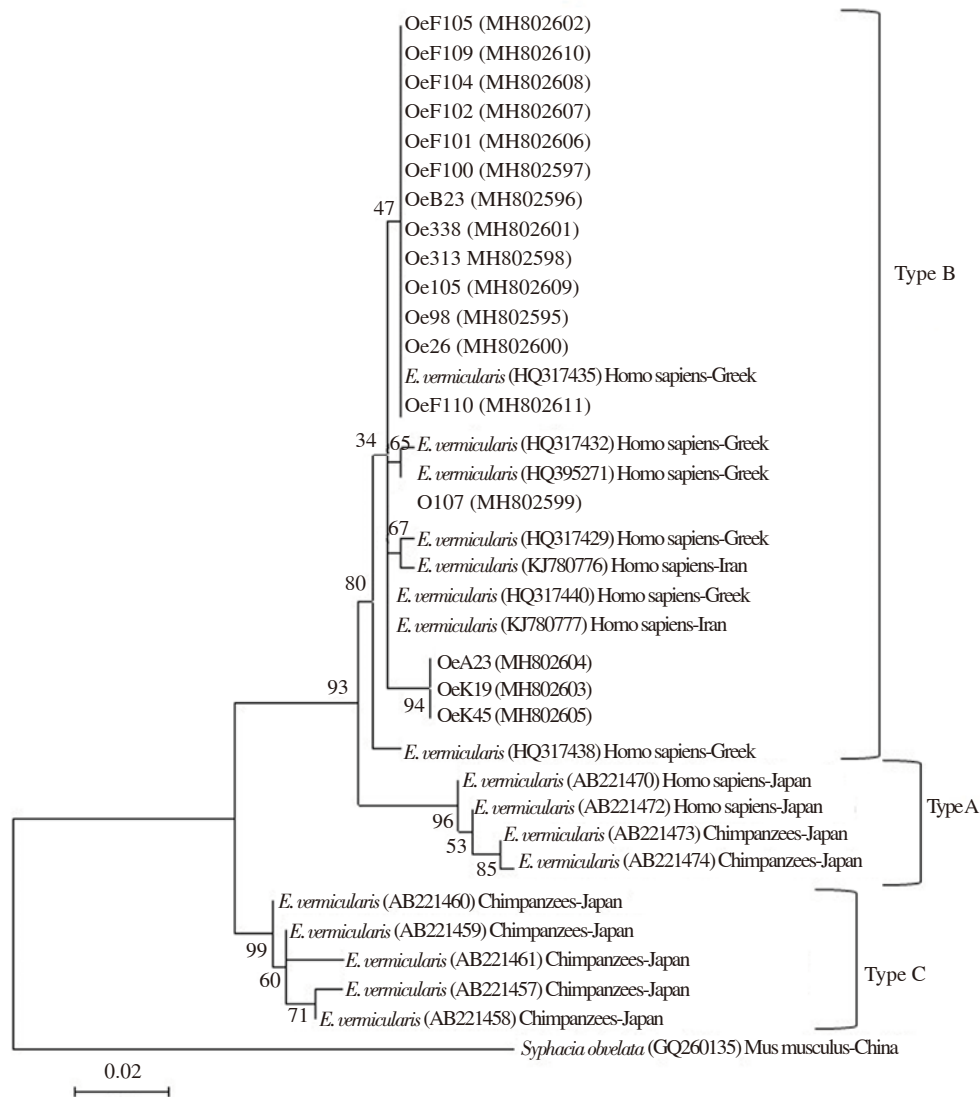


Figure 2. Phylogenetic relationship of *cox1* sequences of *Enterobius vermicularis* isolates obtained in this study and reference sequences retrieved from GenBank, *Syphacia obvelata* (AN: GQ260135) was used as the outgroup.

Sequence analysis was performed for 17 PCR products to characterize the genotype of *E. vermicularis*. BLAST analysis indicated that sequenced isolates belonged to the *E. vermicularis* genotype B. The consensus sequences determined in this study were deposited in GenBank with accession numbers MH802591 to MH802611. The demographic data and accession numbers of the isolates are given in Table 1. The consensus phylogenetic tree indicated that 17 isolates of *E. vermicularis* obtained in the current study based on the *cox1* sequences were divided into three haplotypes and intra-species variation was (0.0%-1.2%). In this study, the *cox1* sequence of one isolate (O107) had a 100% homology with *E. vermicularis* isolated from humans in Iran (Accession no. KJ780777) and Greece (Accession no. HQ317440) (Figure 2).

4. Discussion

The results of the present study indicated a prevalence of 2.7% of *E. vermicularis* infection among kindergarteners in Shiraz and Khorramabad, Iran. Rostami *et al.* reported that the prevalence of *E. vermicularis* was 1.2% in 800 students in the city of Gorgan, Iran, which was lower than the rate in our study[18]. This may be due to the type of the diagnostic method. We used the scotch tape test while they used the direct smear and formalin-ether concentration for diagnosis of *E. vermicularis*. Studies conducted in northern Iran including mild and humid climates reported a higher prevalence rate than that in our study; the weather factors such as humidity and temperature could affect the prevalence of *E. vermicularis*[17,22]. Similar to our study, the prevalence of *E. vermicularis* in kindergartens in Karaj was shown to be 2.3%[19].

Molecular studies on *E. vermicularis* previously conducted in Iran showed the type B in humans[26]. However, the types A, B and C of *E. vermicularis* were reported in humans and chimpanzees[23–27]. The genotype B of *E. vermicularis* was observed in our study, which is similar to the studies performed in Greece, Germany, Denmark and Iran, reporting the genotype B in humans[24–27]. In contrast to our study, Nakano *et al.* reported the genotypes B and A of *E. vermicularis* in chimpanzees and human samples, respectively. They hypothesized that the genotype B of *E. vermicularis* was probably transmitted to chimpanzees from humans and therefore, the type B probably existed in human populations[23]. The genotypes A and B were isolated from both humans and chimpanzees, but the genotype C was reported only in chimpanzees. However, there has been no study to report the genotype C of *E. vermicularis* in humans.

In the present study, the isolates of *E. vermicularis* obtained based on the *cox1* sequences were divided into three haplotypes. A previous study carried out in Iran showed that *E. vermicularis* had two subtypes including B1 and B2 in the northwest of Iran by the *cox1* gene sequencing method[26]. In our study, the *cox1* sequence of one haplotype (the isolates Oe26, Oe98, Oe313, Oe338, OeB23, Oe105, OeF100, OeF101, OeF102, OeF104, OeF105, OeF109, and

OeF110) was identical with *E. vermicularis* isolated from humans in Greece (Accession no. HQ317435). The isolate O107 had a 100% homology with *E. vermicularis* isolated from humans in Iran (Accession no. KJ780777) and Greece (Accession no. HQ317440). The isolates OeK19, OeA23 and OeK45A were identified as the new haplotypes. The genotype B is the only type of *E. vermicularis* which has been reported in Iran[26]. Similar to our study, the intra-species variation was 0-1.2%, the genetic variability was reported among the type B sequences of *E. vermicularis* from 1.2% to 2.1% by Piperaki *et al.*[24].

In conclusion, *E. vermicularis* is still a common intestinal nematode in children. Due to the high prevalence of this parasite in Iran, the prevention and control programs in human populations are necessary. The genotype characterization is important for the planning of prevention and control programs in human and animal communities. As previously reported in other studies, the genotype B of *E. vermicularis* in humans was identified in our study and considered as the only predominant genotype of this nematode in Shiraz and Khorramabad, Iran. For characterization of the genotype of *E. vermicularis* including its haplotypes, comprehensive molecular studies with a large number of *E. vermicularis* isolates from different parts of Iran are necessary.

Conflict of interest statement

We declare that we have no conflict of interest.

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Authors' contributions

FM and SMS designed the study; AT, HM and MS collected the samples; AT, SB, FM and SMS carried out the microscopy method; AT and FM carried out molecular method; FM and AT analyzed and interpreted the data. FM and SMS drafted the manuscript; all the authors read and approved the final version of the manuscript.

References

- [1] Kucik CJ, Martin GL, Sortor BV. Common intestinal parasites. *Am Fam Physician* 2004; **69**(5): 1161-1168.
- [2] Muller R. *Worms and human disease*. 2nd ed. New York: CABI Publishing; 2002.
- [3] Petro M, Lavu K, Minocha A. Unusual endoscopic and microscopic view

- of *Enterobius vermicularis*: A case report with a review of the literature. *South Med J* 2005; **98**(9): 927-929.
- [4] Brown HW, Neva FA. *Basic clinical parasitology*. 5th ed. New York: Appleton century crofts, New York; 1987.
- [5] Serpytis M, Seinins D. Fatal case of ectopic enterobiasis: *Enterobius vermicularis* in the kidneys. *Scand J Urol Nephrol* 2012; **46**(1): 70-72.
- [6] Patel B, Sharma T, Bhatt GC, Dhingra Bhan B. *Enterobius vermicularis*: An unusual cause of recurrent urinary tract infestation in a 7-year-old girl: Case report and review of the literature. *Trop Doct* 2015; **45**(2): 132-134.
- [7] Mallick SK, Sengupta R, Banerjee AK. Unusual presentation of *Enterobius vermicularis* in conjunctival sac. *Trop Doct* 2015; **45**(4): 247-249.
- [8] Choudhury S, Kumar B, Pal DK. *Enterobius vermicularis* infestation of urinary tract leading to recurrent urinary tract infection. *Trop Parasitol* 2017; **7**(2): 119-121.
- [9] Spitale LS, Pizzi RD, Tomas A, Paez Rearte MG, Pizzi HL. *Enterobius vermicularis* enteroparasite role in appendicitis cecal: Enterobiasis and appendicitis. *Rev Fac Cien Med Univ Nac Cordoba* 2017; **74**(3): 277-280.
- [10] Pigac B, Maši S, Maši V. *Enterobius vermicularis* in the endometrium of the uterus: A case report. *Iran J Parasitol* 2017; **12**(4): 638-641
- [11] Gupta B, Jain S. Perianal nodule due to *Enterobius vermicularis*: Cytomorphological spectrum on fine needle aspiration cytology with a review of literature. *Trop Parasitol* 2018; **8**(1): 53-55.
- [12] Dezsényi B, Sárközy L, Kaiser L, Tárkányi K, Nikolova R, Belics Z. Gynecological and obstetrical aspects of *Enterobius vermicularis* infection. *Acta Microbiol Immunol Hung* 2018; **65**(4): 459-465.
- [13] Hammood ZD, Salih AM, Mohammed SH, Kakamad FH, Salih KM, Omar DA, et al. *Enterobius vermicularis* causing acute appendicitis, a case report with literature review. *Int J Surg Case Rep* 2019; **63**: 153-156.
- [14] Markell EK, Voge M, John DT. *Medical parasitology*. 9th ed. London: Saunders Company; 2006.
- [15] Shahabi S. Prevalence of oxyuriasis in student aged 6-13 years in Torkaman port city in the province Mazandaran and comparing the effectiveness of medications mebendazole and piruinium pamoate in *Oxyuris* patients. *Med Purif* 1996; **22**: 17-21.
- [16] Moosazadeh M, Abedi G, Afshari M, Mahdavi SA, Farshidi F, Kheradmand E. Prevalence of *Enterobius vermicularis* among Children in Iran: A systematic review and meta-analysis. *Osong Public Health Res Perspect* 2017; **8**(2): 108-115.
- [17] Soltani S, Salmanzadeh S, Soltani S, Kahvaz MS, Mobarak S, Hezarian M, et al. Prevalence of intestinal parasitic infections in southwest of Iran: A four-year retrospective study. *Infect Disord Drug Targets* 2019. doi: 10.2174/1871526519666191113105043.
- [18] Mohammadi-Meskin V, Hamed Y, Heydari-Hengami M, Eftekhar E, Shamseddin J, Sharifi-Sarasiabi K. Intestinal parasitic infections in Mental Retardation Center of Bandar Abbas, southern Iran. *Iran J Parasitol* 2019; **14**(2): 318-325.
- [19] Abbaszadeh Afshar MJ, Barkhori Mehni M, Rezaeian M, Mohebali M, Baigi V, Amiri S, et al. Prevalence and associated risk factors of human intestinal parasitic infections: A population-based study in the southeast of Kerman Province, southeastern Iran. *BMC Infect Dis* 2020; **20**(1): 12.
- [20] Mahmoudvand H, Badparva E, Khalaf AK, Niazi M, Khatami M, Nazer MR. Prevalence and associated risk factors of intestinal helminthic infections in children from Lorestan Province, western Iran. *Parasite Epidemiol Control* 2020; **9**: e00136.
- [21] Rahimi H, Dehghani M, Norouzi P, Fazli M. Frequency of *Giardia lamblia* and *Enterobius vermicularis* infections in shahroud kindergartens, 2013. *J Ardabil Univ Med Sci* 2015; **15**(1): 7-14.
- [22] Afrakhteh N, Marhaba Z, Mahdavi SA, Garoosian S, Mirnezhad R, Vakili ME, et al. Prevalence of *Enterobius vermicularis* amongst kindergartens and preschool children in Mazandaran Province, north of Iran. *J Parasit Dis* 2016; **40**(4): 1332-1336.
- [23] Nakano T, Okamoto M, Ikeda Y, Hasegawa H. 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* 2006; **100**(1): 51-57.
- [24] Piperaki ET, Spankos G, Patsantara G, Vassalou E, Vakalis N, Tsakris A. Characterization of *Enterobius vermicularis* in a human population, employing a molecular-based method from adhesive tape samples. *Mol Cell Probes* 2011; **25**(2-3): 121-125.
- [25] Ferrero MR, Roser D, Nielsen HV, Olsen A, Negsum P. Genetic variation in mitochondrial DNA among *Enterobius vermicularis* in Denmark. *Parasitol* 2013; **140**(1): 109-114.
- [26] Rahnamaye HHV, Mahami OM, Bazmani A, Miahipour A, Mirsamadi N. Genetic classification and differentiation of *Enterobius vermicularis* based on mitochondrial cytochrome C oxidase (*coxI*) in northwest of Iran. *J Pure Applied Microbiol* 2014; **8**(5): 3995-3999.
- [27] Kubiak K, Dzika E, Pauksto L. Enterobiasis epidemiology and molecular characterization of *Enterobius vermicularis* in healthy children in north-eastern Poland. *Helminthologia* 2017; **54**(4): 284-291.
- [28] Shoup B. Diagnosis and management of pinworm infection. *Prim Care Update Ob/Gyns* 2001; **8**(6): 240-243.
- [29] Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 2011; **28**(10): 2731-2739.