Short Communication

The 18S Ribosomal DNA Sequence of *Strongyloides stercoralis* in Iran

*S Koosha¹, B Kazemi², F Bonyadi¹

¹Parasitology and Mycology Division, Laboratory Sciences Department, Paramedical School, Shaheed Beheshti University M.C., Tehran, Iran
²Cellular and Molecular Biology Research Center, Shaheed Beheshti University, M.C, Tehran, Iran

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Abstract

**Background:** *Strongyloides stercoralis* is a prevalent parasite in some rural areas in the north of Iran. We decided to investigate whether the 18S ribosomal DNA sequence of the parasite in Iran is similar to the findings of the other researchers.

**Methods:** We collected 3514 stool samples from Gilan and Mazandaran, northern Iran, during the year 2005-2006, from which 96 were found infected with *S. stercoralis*. Using Bearman method filariform larvae were isolated. The larval DNA was extracted and subjected for PCR amplification and sequencing.

**Results:** We found 2.73% *S. stercoralis* infection in stool examination. The partially sequence of Iranian *S. stercoralis* 18S rDNA gene was deposited to GenBank at accession number EF062571.

**Conclusion:** The 18S rDNA sequence of *S. stercoralis* in Iran is very similar to the related sequences deposited in GenBank (94-93% identification).

**Keywords:** 18S rDNA, Strongyloides stercoralis, Filariform larvae, PCR, Iran

Introduction

*Strongyloides stercoralis* is an important human pathogenic parasite. It has the capacity of the internal and external autoinfection and altering from free-living to parasitic life cycle. The ability to multiply in its host and the environment causes *Strongyloides* to survive and persist. It is prevalent in people who live in tropical and subtropical areas with almost heavy rainfall (1) but also occurs at low prevalence (4.9%) in some rural areas in the north of Iran (2). *S. stercoralis* is transmitted by contact with contaminated moist soil. The infection is mostly asymptomatic but when the normal life cycle of the parasite changes; it can have considerable potential for causing disease (especially in certain immunosuppressed patients who usually suffer from severe complicated strongyloidosis) and death. Diagnosis is performed by observation of the characteristic larvae in the stools using either direct, concentration or cultivation methods, which are not always reliable (3). Serodiagnosis is another choice for diagnosis (4), and today molecular biological methods are applied to detect specific DNA of *S. stercoralis* with ribosomal DNA sequences used in some studies to design radioactive DNA probes or specific PCR primers (5, 6).

*Corresponding Author: Soheila_koosha@yahoo.com*
Due to the lack of any significant molecular study on this parasite in Iran, we decided to investigate if the 18S ribosomal DNA sequence of the parasite in Iran is similar to the findings of the other researchers. We have done a survey in two neighboring endemic provinces in the north of Iran (Gilan and Mazandaran) (7), located beside the Caspian Sea with temperate climate.

**Material and Methods**

**Sampling**

Gilan and Mazandaran provinces have 2389195 and 2796120 population, respectively (8). Based on statistician’s advice, during years 2005-2006 we chose sixteen primary health care units (7 in Gilan and 9 in Mazandaran) we collected 3514 stool samples by using random stratified sampling. Initially using direct method we examined the samples and then by using the Bearman method we isolated the filariform larvae in the positive samples cultured on charcoal (3).

**DNA extraction**

The 50 *S. stercoralis* larvae were rinsed by PBS buffer three times to remove remained feces, then suspended with 1 ml of lyses buffer (10mM Tris, 10 mM EDTA, 150 mM NaCl and 2% SDS/2% Triton -X100) and submitted to DNA extraction through phenol-chloroform method. DNA was precipitated with ethanol and dissolved it in sterile deionized water (9).

**PCR Amplification**

We designed a set of primers for nested PCR based on GenBank accession number AJ47023. The nest I primers (SsF 5’- ACA CGG TAA ATA TTT TAG TTG – 3’ and SsR 5’- CTA AAT CAT GAA AGA GCT ATC -3’) amplified a *Strongyloides* 18S rDNA gene specific PCR product of 1092 bp and Nest II primer (SsF2 5’- GCT AAT ACA CGC TAT TTA TAC-3’ and SsR2 5’- GTT GAG TCA AAT TAA GCC GC-3’) a specific PCR product of 975 bp. The PCR mixture contained: 0.25 mM dNTP, 1.5 units Taq DNA polymerase (CinnaGen, Iran), 20 Pico moles each of the forward and reverse primers, 1x PCR buffer, 1.5 mM MgCl2, 100ng template DNA (1 micro liter of nest I PCR product for PCR II initiation) and distilled water up to 50µl. PCR cycling parameters included initial predenaturation at 94 °C for 5 min. The incubation cycles consisted of denaturation at 94 °C for 30 sec, primer annealing at 60 °C for 30 sec and extension at 72 °C for 30 sec, repeated for 30 cycles with a final incubation at 72 °C for 5 min (10).

**Electrophoresis**

PCR products were submitted to electrophoresis using 1% agarose gel and were stained with ethidium bromide (0.5 µg/ml). The DNA bands were visualized under ultraviolet light (UV transilluminator) (11).

**Sequencing**

The 975 bp PCR product was purified by DNA purification kit (Fermentas Cat. No. k0513) and was subjected to sequencing by dideoxy chain termination method using nest I forward primer (12).

**Results**

Ninety six positive samples were detected and cultured for recovery from *S. stercoralis’* larvae (2.73%). Genomic DNA was extracted from lysed filariform larvae and subjected to 18S rDNA gene amplification using PCR method. The nested-PCR product was electrophoresed on agarose gel shown in Fig. 1. PCR product of *S. stercoralis* 18S rDNA was purified, sequenced and deposited to GenBank at accession number EF062571. With Blast software it was compared with the other deposited 18S rDNA genes of *S. stercoralis* from the other countries (Fig. 2). There were lots of similarities (94-93% homology) between 18S rDNA sequence of *S. stercoralis* in Iran and the corresponding sequences of the other geographical areas.
**Fig 1.** 1% agarose gel electrophoresis
Lane a: 975 bp as PCR product of 18S rRNA *Strongyloides stercoralis*
Lane b: 100 bp DNA ladder marker

**Fig. 2:** Comparison between the 18S rDNA sequence of Iranian *Strongyloides stercoralis* with the high similarities accession numbers in Genbank
Fig. 2: Continued…

Fig. 3: Slanted distance tree of the 18S rDNA sequence of Iranian Strongyloides stercoralis and the high similarities accession numbers in Genbank (using blast pairwise alignments)
Discussion

The prevalence of *S. stercoralis* infection in tropical and subtropical areas with its alarming health and economic consequence has led researchers to perform studies on epidemiologic aspects, diagnostic methods, definition of gene markers and the control of the infection (13). Moreover, in molecular biology and genetic research it has been found advantageous to use the genus *Strongyloides* because of its altering parasitic and free-living generations and vice versa (14). But since morphologically it is not possible to make distinction among different *Strongyloides* species’ larvae, some of which may cause disease in human beings, DNA sequence analysis has been used to make accurate diagnosis (15). Consequently, sequencing of small subunit ribosomal DNA genes has become the “gold standard” (16), and the 18S rDNA has a variation in nucleotide sequences among *Strongyloides* spp. which is a “suitable species marker” (15) for genetic variability analysis in molecular epidemiology.

This is the first report of *Strongyloides* 18S rRNA gene sequence from Iran. But there are some GenBank accession numbers on it from Iran, with other fragments of rDNA gene (ITS1 region, accession numbers: EF653264-66 and EF545004) (17). The parasite 18S rDNA gene sequence at GenBank accession numbers AF279916, AJ417023 (from UK) (18, 19), AB453314, AB453316, AB453315 (from Japan) (15) and M84229 (from Australia) (20) were used for comparison in this study (Fig. 2). The highest degree of similarity between the gene sequence in this article and the already mentioned ones are 94% for the first threes and 93% for the others. As the slanted distance tree shows, all the sequences branched from a unique source but the second branch indicates that with the Iranian sequence as an exception, the others all originated from the same source (Fig. 3). Probably these results are due to their different geographical regions. “The nucleotide arrangement presumably reflects the process of geographical dispersal and adaptation to the host” (15). It seems that it is required to perform further investigations on 18S rDNA gene sequence of *S. stercoralis* to find out more about this gene in other parts of Iran.

In conclusion, the sequence of Iranian *S. stercoralis* 18S rDNA gene has a high degree of similarity (94-93%) with the related sequences previously deposited in GenBank.

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