Screening of *Strongyloides stercoralis* Infection in High-Risk Patients in Khuzestan Province, Southwestern Iran

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Research

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Abstract

Background: Strongyloidiasis, one of the neglected tropical diseases (NTDs), can be fatal in immunocompromised patients. Available data on *Strongyloides stercoralis* infection in high-risk patients in Iran; however, is limited. The aim of the present study was to determine the prevalence of *S. stercoralis* infection and associated risk factors among high-risk patients, as well as to evaluate the accuracy of the diagnostic tests used in the diagnose of *S. stercoralis* infection.

Methods: This cross-sectional study was performed during 2019 to 2020 among 300 high-risk patients in Khuzestan Province, southwestern Iran. Patients with autoimmune diseases, uncontrolled diabetes, HIV/AIDS, cancer, organ transplant, hematologic malignancy, asthma and chronic obstructive pulmonary disease (COPD) were examined using direct smear examination, formalin-ether concentration, Baermann funnel technique, agar plate culture, and ELISA test. Culture-positive samples were confirmed by PCR amplification and sequencing of the nuclear *18S* rDNA (*SSU*) hypervariable region (HVRIV) of the parasite.

Results: The prevalence of *S. stercoralis* infection was 1%, 1.3%, 2%, 2.7%, and 8.7% using direct smear examination, formalin-ether concentration, Baermann funnel technique, agar plate culture, and ELISA test, respectively. All culture-positive samples were confirmed by *SSU*-PCR. According to the results, the most sensitive test was ELISA, with 100% sensitivity, followed by Baermann funnel technique with sensitivity of 75%. Direct smear examination, formalin-ether concentration technique, and Baermann funnel technique had the highest PPV (100%), while ELISA test had the highest NPV (100%). Eosinophilia significantly was observed in patients whose culture test were positive (7/8; p= 0.001). Of these, 5 patients had a history of asthma and COPD. In the present study, the majority of the positive cases by the agar plate culture had a history of prolonged exposure to soil, history of asthma and COPD, and were over the ages of 60. In two cases, *S. stercoralis* larva was observed 6 months and 8 months after treatment with albendazole**.**

Conclusions: Given that the ELISA test had the highest NPV, screening of all high-risk patients for *S. stercoralis* infection in endemic areas is recommended prior to starting corticosteroid therapy with ELISA test. Ivermectin should be available to the strongyloidiasis patients in the endemic areas.

Background

Strongyloidiasis is a soil-transmitted helminthiasis (STH), caused mainly by the species *Strongyloides stercoralis.* This intestinal nematode, with a prevalence nearly 30 – 100 million people worldwide, is one of the neglected tropical diseases (NTD) [1, 2]. *Strongyloides stercoralis* infection generally occurs in tropical and subtropical countries, particularly in areas with warm and humid climate [3, 4]. Although this nematode has a complex life cycle, the most frequent infection rout is percutaneous entry of the filariform larvae [4]. Clinical manifestations of strongyloidiasis vary from asymptomatic or mild gastrointestinal and/or cutaneous symptoms in healthy people [5] to severe clinical symptoms in immunocompromised patients which lead to hyperinfection syndrome or disseminated strongyloidiasis [6]. Various factors, including corticosteroid drugs, human T-cell lymphotropic virus type 1 (HTLV-1)

infection, transplant, human immunodeficiency virus (HIV) infection, malnutrition, diabetes mellitus (DM), chronic obstructive pulmonary disease (COPD), and lung disease impair immune responses and put the patient at risk for the severe strongyloidiasis [7, 8].

Several parasitological and serological tests are available to diagnose *S. stercoralis* infection. Among those, the culture technique is currently considered the clinical gold standard. However, this technique has limitations due to the need for fresh stool, time-consuming, and inability to distinguish between *S. stercoralis* and hookworms larva [9]. Among the serological tests, enzyme-linked immunosorbent assay (ELISA) is increasingly being used to overcome the limitations of parasitological techniques. However, this method has also limitations, including cross reaction with other helminthic infections and difficulty in distinguishing between current and past *S. stercoralis* infection [5].

Iran, one of the tropical and subtropical regions, is an endemic area for *S. stercoralis*; however, most reports belong to northern and southern parts of the country [10, 11, 12]. In a study conducted by Rafiei et al. in 2016, the seroprevalence of *S. stercoralis* was reported 14.4% in immunocompromised patients from southwestern Iran [10]. Since many strongyloidiasis cases are asymptomatic and conventional parasitological examinations are not sufficiently sensitive, *S. stercoralis* infection is frequently under-diagnosed [8]. However, the potential of uncontrolled multiplication and life-threatening dissemination of *S. stercoralis* larvae in immunocompromised patients can lead to mortality rates to 85% [13]. Despite the importance of early detection of *S. stercoralis* in immunocompromised patients, very few studies have investigated strongyloidiasis in high risk groups, particularly patients who receive immunosuppressive drugs in Iran [10, 14, 15, 16]. In addition, available data on strongyloidiasis in Iranian high-risk patients is limited to the few studies conducted with only one or two diagnostic methods. The main aim of the present survey was to assess the frequency of *S. stercoralis* in high-risk patients in Khuzestan Province, southwestern Iran, using parasitological, serological, and molecular methods. Furthermore, the study was aimed to evaluate the accuracy of the diagnostic tests used in the diagnosing of *S. stercoralis* infection in high-risk patients.

Methods

Study area

Khuzestan Province (31.3273°N 48.6940°E), one of the 31 provinces of Iran, located in the southwest of the country, bordering Iraq and the Persian Gulf. The majority of the province has a mild winter and very hot summer. However, some parts of Khuzestan Province have humid summers. Northern parts of the province have cultivation of wheat, barley, rice, and sugar cane, while southern parts, such as Abadan and Khoramshahr Counties produce date palm.

Study population, study design and clinical assessment

This cross-sectional study was designed to evaluate prevalence of *S. stercoralis* among patients referred to 17 Shahrivar (Abadan) and Shahid Baghaee (Ahvaz) hospitals from June 2019 to March 2020. The selected counties were chosen based on previous studies [10, 17] and hospitals reports. The sample size was determined using previous study with a prevalence rate of 14.4% and confidence interval of 95% [10]. The calculated sample size was 296 patients, thus 300 patients enrolled in the study. Simple random sampling of patients was carried out according to the number of high-risk patients referred to each hospital. Inclusion criteria were high-risk groups, including patients with autoimmune diseases, rheumatoid arthritis, uncontrolled diabetes (Fasting Blood Sugar level > 300 mg/dl or HbA1c > 9), HIV/AIDS, cancer, organ transplanted cases, patients receiving corticosteroids, malnourished patients, asthma and chronic obstructive pulmonary disease (COPD) patients. Children under five-years-old, patients who had anti-helminth therapy in the past six months, patients living outside the study area, patients who had other parasitic diseases other than *S. stercoralis* infection, and those who did not provide stool specimen were excluded from the study.

Socio-demographic data (age, gender, and residency area) and clinical characteristics of all patients were collected by a standardized questionnaire. A clinical assessment was conducted by two internal medicine and oncologist at 17 Shahrivar (Abadan) and Shahid Baghaee (Ahvaz) hospitals, respectively.

Laboratory investigation procedures

Clean plastic flasks were provided to the patients or their companions and fecal samples were collected the following day. Two mL venous blood sample was obtained from each patient for serological test. After rapid transfer to the Parasitology Department of Ahvaz Jundishapur University of Medical Sciences, the sera were stored at − 20 °C for ELISA test and the fecal samples were processed immediately for further examinations

Direct smear examination

The fecal samples were examined microscopically for detecting *S. stercoralis* larva at 100X and 400X magnifications using Saline/Lugol's iodine staining method. To increase the sensitivity, three smears of each sample were examined.

Formalin-ether concentration technique

Fecal samples were analyzed by formalin-ether concentration technique as described previously [18]. Approximately 1 mg of each specimen was suspended in 10 mL saline (0.9%) and was passed through a four layers gauze. The suspension was centrifuged at 700 × g for 5 min at room temperature. After discarding the supernatant, 7 mL formalin (10%) and 3 mL diethyl ether were added to the sediment. The

suspension was shaken vigorously for 1 min and centrifuged at 700 × g for 5. The top three layers were removed and sediment was examined microscopically at 100X and 400X magnifications [18].

Baermann funnel technique

Approximately 4–5 g fresh fecal sample was placed on the center of a 4 layers gauze and it was closed using a rubber band. The specimen was immersed in a funnel filled with lukewarm water that had blocked its bottom. It was leaved for 16–18 h at room temperature. The collected suspension was centrifuged at 500 × g for 2 min then the sediment was analyzed using normal saline at 100X magnification.

Agar plate culture

For the agar plate culture, approximately 3 g of fresh fecal sample was placed on the center of nutrient agar plate. The plates were sealed with cellulose tape and placed into a box at room temperature (25–30 ^{o}C) for seven days. On days 2 to 7, the surface of plates was washed with normal saline solution and centrifuged at 1500 rpm for 5 min to collect larva and adult forms of *S. stercoralis.* For discriminating *S. stercoralis* and hookworms larva morphologically the sediment was examined under microscopic magnification of 400X. Based on previous studies, the agar plate culture method was considered as the most efficient technique.

Enzyme-Linked Immunosorbent Assay *(*ELISA*)*

ELISA test was conducted by IgG ELISA (NOVA TEC Immunodiagnostica GmbH, Dietzenbach, Germany) kit using recombinant immunodiagnostic antigen (NIE) according to the manufacture instructions. The absorbance values were measured at 450 nm using Dynex DS2® automated ELISA reader. The results were calculated according to NovaTec Units (NTU). The Cut-off was determined 10 NTU. The values above 11 NTU were reported positive, between 9 – 11 NTU and below 9 NTU were reported equivocal and negative, respectively.

Molecular analysis

DNA extraction was performed on agar plate culture-positive samples as described previously [19]. The collected larva and adult forms were frozen for 5 min in liquid nitrogen and were thawed for 5 min in a boiling water bath. Freezing and thawing step was repeated five times. The lysate was stored at − 20^{o}C until use.

Confirmation of the *S. stercoralis* infection was performed by a PCR protocol to amplify a 712- bp fragment of the nuclear *18S* rDNA (*SSU*) hypervariable region (HVRIV) of the parasite as described before [19]. The PCR was carried out in a 20-µl reaction volume containing 10 µL of 2 × Master Mix RED

(Ampliqon-Biomol, Hamburg, Germany), 0.5 µL of each forward and reverse primers, 7 µL nuclease-free water, and 2 µL of template DNA. The 712 bp fragment was amplified using forward primer 18SP4F (5´–GCGAAAGCATTTGCCAA– 3´) and reverse primer 18SPCR (5´–ACGGCCGGTGTGTAC– 3´) as described by Hasegawa et al. (2009) [20]. The PCR cycling was as follows: an initial denaturation at 95 °C for 30 s, followed by 35 cycles of 95 °C for 20 subtype (denaturation), 55 °C for 15 s (annealing), 68 °C for 90 s (extension), and a final extension of 68 °C for 5 min. The PCR product was used for sequencing using primer ZS6269 (5´–GTGGTGCATGGCCGTTC– 3´) at Pishgam biotech Co. (Tehran, Iran) [21]. The obtained sequences were assembled using Chromas version 2.1 and were compared with sequences previously deposited at the National Centre for Biotechnology Information (NCBI) using the BLAST tool (http://www.ncbi.nlm.nih.gov/blast).

Statistical analysis

Data analysis was conducted using SPSS 22 software (SPSS Inc., Chicago, IL, USA). To compare proportions, Chi-square test was used. The agar plate culture was compared with other coproparasitological and ELISA methods using Chi-square and Fisher's exact test. Receiver Operating Characteristic (ROC) curve was used to evaluate the coproparasitological and ELISA test compared to the agar plate culture the clinical standard method for diagnosis of *S. stercoralis* larva and adult forms. Moreover, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (PNV) of the diagnostic methods were estimated. The Kappa test was used to assess concordance between the agar plate culture test and other diagnostic tests.

Results

Patient characteristics

Of the 300 participated patients in the study, 27.3% had history of uncontrolled diabetes, 34.0% including 17.7% autoimmune diseases and 16.3% asthma and chronic obstructive pulmonary disease (COPD) receiving steroids, 26.3% had malignant diseases (cancer and leukemia), and 12.4% had other diseases (AIDS, solid organ transplantation, and malnutrition). Regarding the gender, 144 (48.0%) were male and 156 (52.0%) were female. The age range of the patients was 5 – 93 years (mean 54.71; SD: 18.1). Of these, 41.7% (125/300) were in the age group above 60 years old. Regarding the place of residence, 67.3% (202/300) were from urban and 32.7% (98/300) were from rural areas. According to the Table 1, 43.0%, 10.7%, and 7.3% of the patients were housewife, farmer, and fisherman, respectively. Gastrointestinal and pulmonary symptoms were reported by 32.7% and 12.7%, respectively. In 9.0% patients both gastrointestinal and pulmonary symptoms were observed. At the time of referral, 51 (17.0%) of the patients had diarrhea (Table 1). Data on the eosinophils was obtained by examining the patient files. Of the 175 patients whose CBC tests were performed, 46 (26.3%) had eosinophil count above 10.0%. Of these, the maximum percentages (64.0%) belonged to the patients treated with steroids.

Table 1
Socio-demographic and clinical characteristics of patients according to underlying disease.

Characteristics	All patients No.= 300	Uncontrolled diabetes No.= 82	Malignant diseases No.= 79	Steroid therapy No.= 102	Other diseases No.= 37
	n (%)	n (%)	n (%)	n (%)	n (%)
Age (years)					
< 20	16 (5.3)	1 (6.3)	13 (81.3)	2 (12.5)	0 (0)
20 – 40	45 (15.0)	3 (6.7)	17 (37.8)	19 (42.2)	6 (13.3)
41 – 60	114 (38.0)	40 (35.1)	29 (25.4)	28 (24.6)	17 (14.9)
60+	125 (41.7)	38 (30.4)	20 (16.0)	53 (42.4)	14 (11.2)
Gender					
Male	144 (48.0)	32 (22.2)	50 (34.7)	37 (25.7)	25 (17.4)
Female	156 (52.0)	50 (32.1)	29 (18.6)	65 (41.7)	12 (7.7)
Living area					
Rural	98 (32.7)	34 (34.7)	24 (24.5)	26 (26.5)	14 (14.3)
Urban	202 (67.3)	48 (23.8)	55 (27.2)	76 (37.6)	23 (11.4)
Clinical symptoms					
Gastrointestinal	98 (32.7)	41 (41.8)	24 (24.5)	13 (13.3)	20 (20.4)
Pulmonary	38 (12.7)	4 (10.5)	3 (7.9)	30 (79.0)	1 (2.6)
Gastrointestinal/ Pulmonary	27 (9.0)	0 (0.0)	4 (14.8)	22 (81.5)	1 (3.7)
Asymptomatic	137 (45.6)	37 (27.0)	48 (35.1)	37 (27.0)	15 (10.9)
Diarrhea					

Characteristics	All patients No.= 300	Uncontrolled diabetes No.= 82	Malignant diseases No.= 79	Steroid therapy No.= 102	Other diseases No.= 37
	n (%)	n (%)	n (%)	n (%)	n (%)
Yes	51 (17.0)	17 (33.3)	11 (21.6)	11 (21.6)	12 (23.5)
No	249 (83.0)	65 (26.1)	68 (27.3)	91 (36.6)	25 (10.0)
Soil exposure					
Low	221 (73.7)	58 (26.3)	57 (25.8)	79 (35.7)	27 (12.2)
High	79 (26.3)	24 (30.4)	22 (27.8)	23 (29.1)	10 (12.7)

Strongyloides stercoralis prevalence, ROC curves, Predictive values, and Concordance

The observed prevalence of *S. stercoralis* was 1% (3/300; 95% confidence interval: 1–1.02%) using direct smear examination. Using formalin-ether concentration, Baermann funnel technique, agar plate culture, and ELISA the prevalence was 1.3%, 2%, 2.7%, and 8.7%, respectively (Fig. 1; Table 2). Compared to the agar plate culture, the direct smear examination ROC had an AUC (area under the curve) of 0.688 with 37.5% sensitivity. Furthermore, when the other tests were compared to the agar plate culture, the formalin-ether concentration Roc had an AUC of 0.750 with 50% sensitivity, the Baermann funnel technique had an AUC of 0.875 with 70% sensitivity, and the ELISA test had an AUC of 0.955 with 100% sensitivity and 93.8% specificity (Fig. 2). The ELISA test was founded as the most sensitive test, with a sensitivity 100%, followed by Baermann funnel technique with sensitivity of 75%. According to the data collected in Table 3, the direct smear examination, formalin-ether concentration technique, and Baermann funnel technique had the highest PPV (100%), while ELISA test had the highest NPV (100%). Baermann funnel technique showed the highest agreement with the agar plate culture (0.854) followed by formalin-ether concentration (0.661). Data summarized in Table 4 indicated that the occupation of patients (p = 0.002), type of underlying disease (p = 0.032), clinical symptoms (0.017), eosinophilia (0.001), soil exposure (0.002), and diagnostic method (p = 0.001) were significantly associated with the presence of *S. stercoralis* larva by the agar plate culture. The obtained results by the culture indicated that no significant differences were observed between gender (p = 0.162), residency (p = 0.445) and infection. However, of the 8 positive cases by the agar plate culture, 7 patients were from Abadan County. Of the eight positive

cases by agar plate culture, two cases were positive 6 and 8 months after treatment, respectively, by the agar plate culture. A marginal trend toward significance (p = 0.052) was founded between the age groups and *S. stercoralis* infection. Among the clinical factors, eosinophilia significantly was observed in patients whose culture test were positive (7/8; p = 0.001). Moreover, of the 26 ELISA positive cases, 17 (65.4%; p = 0.001) cases had eosinophilia. All culture-positive samples confirmed by PCR (Fig. 3).

Table 2
Frequency of *Strongyloides stercoralis* larva in the stool samples of 300 patients studied, according to coproparasitological and serological methods

Techniques	Uncontrolled diabetes No.= 82	Malignant diseases No.= 79	Steroid therapy No.= 102	Other diseases No.= 37	*P*
	n (%)	n (%)	n (%)	n (%)	
Direct smear examination					
Positive	1 (33.3)	0 (0.0)	2 (66.7)	0 (0.0)	0.539
Negative	81 (27.3)	79 (26.6)	100 (33.7)	37 (12.4)	
Formalin-ether concentration technique					
Positive	1 (25.0)	0 (0.0)	3 (75.0)	0 (0.0)	0.311
Negative	81 (27.4)	79 (26.7)	99 (33.4)	37 (12.5)	
Baermann funnel technique					
Positive	1 (16.7)	1 (16.7)	4 (66.7)	0 (0.0)	0.369
Negative	81 (27.6)	78 (26.5)	98 (33.3)	37 (12.6)	
Agar plate culture					
Positive	1 (12.5)	1 (12.5)	6 (75.0)	0 (0.0)	0.096
Negative	81 (27.7)	78 (26.7)	96 (32.9)	37 (12.7)	
Enzyme-linked immunosorbent assay					
Positive	11 (42.3)	3 (11.5)	9 (34.7)	3 (11.5)	0.287
Equivocal	1 (25.0)	2 (50.0)	0 (0.0)	1 (25.0)	
Negative	70 (25.9)	74 (27.4)	93 (34.5)	33 (12.2)	

Table 3

Sensitivity, specificity, positive predictive value, negative predictive value, and concordance between the agar plate culture and other diagnostic test used in this study.

Diagnostic tests	Sensitivity%	Specificity%	PPV%	NPV%	Concordance
DSE	37.5	100	100	98.3	0.539
FECT	50	100	100	98.6	0.661
BFT	75	100	100	99.3	0.854
ELISA	100	93.8	30.8	100	0.448

DSE: Direct smear examination; **FECT**: Formalin-ether concentration technique; **BFT**: Baermann funnel technique; **ELISA**: Enzyme-linked immunosorbent assay, **PPV**: positive predictive value; **NPV**: Negative predictive value.

Table 4
Socio-demographic and clinical characteristic associated with *Strongyloides stercoralis*, according to the agar plate culture.

Characteristics		Positive (%)	Negative (%)	*P*
Gender	Male	6 (4.1)	139 (95.9)	0.162
	Female	2 (1.3)	153 (98.7)	
Age (years)	< 20	0 (0.0)	16 (100.0)	0.052
	20 – 40	1 (2.2)	44 (97.8)	
	41 – 60	0 (0.0)	114 (100.0)	
	60+	7 (5.6)	118 (94.4)	
Living area	Rural	4 (4.1)	94 (95.9)	0.445
	Urban	4 (2.0)	198 (98.0)	
Occupation	Housewife	2 (1.6)	127 (98.4)	0.002
	Farmer	4 (12.5)	28 (87.5)	
	Worker	2 (7.7)	24 (92.3)	
	Fisherman	0 (0.0)	22 (100.0)	
	Student	0 (0.0)	13 (100.0)	
	Other	0 (0.0)	78 (100.0)	
Diseases	Cancer	0 (0.0)	46 (100.0)	0.032
	Uncontrolled diabetes	1 (1.2)	81 (98.8)	
	Autoimmune diseases	1 (1.9)	52 (98.1)	
	Hematologic malignancy	1 (3.0)	32 (97.0)	
	Asthma& COPD	5 (10.2)	44 (89.8)	
	Transplant	0 (0.0)	9 (100.0)	
	Other	0 (0.0)	28 (100.0)	
Clinical symptoms	Gastrointestinal	1 (1.0)	97 (99.0)	0.017
	Pulmonary	2 (5.3)	36 (94.7)	
	Gastrointestinal/ Pulmonary	3 (11.1)	24 (88.9)	
	Asymptomatic	2 (1.5)	135 (98.5)	
Eosinophilia	Yes	7 (15.2)	39 (84.4)	0.001

Characteristics		Positive (%)	Negative (%)	*P*
	No	1 (0.8)	128 (99.2)	
Soil exposure	Low	2 (0.9)	219 (99.1)	0.002
	High	6 (7.6)	73 (92.4)	
Diagnostic method	DSE	3 (37.5)	5 (62.5)	0.001
	FECT	4 (50.0)	4 (50.0)	
	BFT	6 (75.0)	2 (25.0)	
	ELISA	8 (100.0)	0 (0.0)	

Discussion

This cross-sectional study was performed on high-risk patients to evaluate the prevalence of *S. stercoralis* infection using parasitological, serological, and molecular methods. Since previous studies have demonstrated the sensitivity of 90–97.5% for the agar plate culture [22, 23], it was considered the reference test in the present study. In this study, the prevalence of *S. stercoralis* was 8.7% with the ELISA test. The sensitivity and specificity of the ELISA test were 100% and 93.8%, respectively. The seroprevalence observed by the ELISA test was 3.2 greater than the agar plate culture. Among the parasitological methods, the formalin ethyl acetate concentration technique is more used in routine fecal examinations, because the results can be achieved more quickly [5]. However, it is difficult to recognize the dead larvae by the formalin ethyl acetate at low magnification [24]. Baermann funnel technique and agar plate culture are other diagnostic methods used in *S. stercoralis* detection. Although both techniques are more sensitive in detecting *S. stercoralis* than the direct method, they are cumbersome and time-consuming procedures [5]. Nevertheless, the agar plate culture is used as a more efficient technique in clinical laboratories to diagnose *S. stercoralis* [23]. In this study, prevalence of *S. stercoralis* with the agar plate culture (2.7%) was lower than the ELISA test (8.7%), but it was higher compared to the formalin-ether concentration technique (1.3%), Baermann funnel technique (2%), and direct smear examination (1%). The obtained results revealed that the direct smear examination had the lowest sensitivity in diagnosing *S. stercoralis* (37.5%). These findings match those observed in previous studies [3, 22, 25]. A single stool examination fails to diagnose in up to 70% of the strongyloidiasis cases [26]. Serial stool examinations in three consecutive days can increase the chance of diagnosis by direct smear examination. According to a study conducted by Khieu et al. (2013), prevalence of *S. stercoralis* infection was increased from 15.9–21.6% by analyzing three stool samples [27]. In chronic strongyloidiasis, sensitivity of single stool examination in detecting *S. stercoralis* in symptomatic patients is approximately 50%, while in asymptomatic patients probably is less [26, 28]. The low parasite load and the periodic excretion of *Strongyloides* larvae make it difficult to detect microscopically [29]. It should be noted that sampling in consecutive days, especially in patients with underlying diseases, will be very difficult and impractical, thus the solution is to use more sensitive methods.

In the present study, the ELISA test demonstrated a higher prevalence rate than the coproparasitological methods; however the prevalence observed was lower compared to those reported by Rafiei et al., in the same region [10]. This discrepancy might be related to the cross reactivity in patients with other STH infections and/ or the difference in the sensitivity of the ELISA kits. Another possible explanation for this result might be that we used NovaLisa *Strongyloides*, ELISA Kit (NovaTec Immunodiagnostica, Germany), which it uses a recombinant antigen (NIE). ELISA test has limitations of inability to identify between current and past infection and lower sensitivity in diagnosing strongyloidiasis in patients with hematologic malignancies and/ or infected with HTLV-1 [5]. To overcome these limitations, *Strongyloides*-specific recombinant antigens, such as NIE, SsIR, and rSs1a have been used in ELISA kits [5, 29, 30, 31]. The coproantigen ELISA has also demonstrated promising results in detecting current infections [32]

According to the obtained results (Table 3), the direct smear examination, formalin-ether concentration, and Baermann funnel technique were the tests with the highest specificity. Given that these tests showed the highest PPV, they could be used routinely for diagnosis of *S. stercoralis* larva or epidemiological studies. ROC analysis showed that ELISA test with an AUC 0.955 and sensitivity of 100% was the most sensitive test in diagnosing strongyloidiasis.

The prevalence observed in this study was not significantly different between males and females by the agar plate culture; however, males showed a higher prevalence (4.1%) than females (1.3%). This may be due to the fact that some men are more exposed to contaminants due to working outside the home. These findings are consistent with those of Luvira et al. [9] who found higher infection rates in males, but differ from those of other studies [10, 22]. Although we could not find a significant difference between rural and urban areas ($p = 0.445$), previous studies have reported that living in rural areas and prolonged exposure to contaminated soil may put humans at *S. stercoralis* infection risk [3, 33]. In Iran, migration from rural to urban areas has increased in recent years. It is possible that some patients who live in the urban areas were infected during their residency in the rural areas. In our study, of the eight positive cases by the agar plate culture, 75% had a history of prolonged exposure to soil. This result may be explained by the fact that walking barefoot is common among some rural residents of Khuzestan Province. Another possible explanation is the occupation exposure of some patients to contaminated soil. According to the occupational data in Table 4, a significant association was found between occupation and infection ($p = 0.002$). The highest infection rate (50.0%), belonged to the farmers. In the tropics, warm moist temperatures, lower socioeconomic, and poor hygienic conditions increase risk of *S. stercoralis* infection [34]. Age-related findings revealed that the most of the diagnosed cases by the agar plate culture (87.5%), and ELISA test (57.7%) were patients over 60 years old. These results differ from previous published studies [3, 10], but agree with the findings of other studies [35, 36, 37]. This result may be explained by the fact that the *S. stercoralis* has a unique life cycle that allows autoinfection [5]. Therefore, in elderly population that immune system is weakened [37], parthenogenesis by the adult female may lead to accelerated autoinfection and clinical presentations [5]. Clinical manifestations such as gastrointestinal and pulmonary symptoms were associated with the agar plate culture positive ($p = 0.017$), but not with the ELISA test ($p = 0.311$). The ability of the parasite to replicate might lead to persistence within a host for decades. This discrepancy between ELISA results and clinical symptoms could be attributed to the

past *S. stercoralis* infection. Chronic strongyloidiasis is often asymptomatic, but in acute strongyloidiasis main complications are gastrointestinal and pulmonary symptoms. Peripheral eosinophilia is common during acute infection (75%-80%) but intermittent or in some cases up to 75% in chronic infection, and absent in severe strongyloidiasis and the immunocompromised patients [38, 39]. In our study, 87.5% of the positive cases by the agar plate culture had eosinophil count above 10.0%. The significant association between infection and eosinophilia ($p = 0.001$) might be related to the fact that *S. stercoralis* female worm live within the submucosa of the gut and thus the eosinophilic response may occurs at high level [8]. Should be mentioned that out of the seven positive cases who had eosinophilia, 5 patients had a history of asthma and COPD. Therefore, this finding might be related to their underlying diseases. In a study conducted by Ashrafi et al. (2010) from Gilan Province, Northern Iran, out of 150 patients with undiagnosed eosinophilia, 42% were diagnosed with strongyloidiasis by the formalin-ether and the Kato-Katz techniques [40].

Statistical analysis showed that the Baermann funnel technique had the highest agreement with the agar plate culture (0.854), so that it was detected 75% of the positive cases by the agar plate. This finding is in agreement with Hailegebriel et al. (2017) findings which showed 75% of *S. stercoralis* infection was diagnosed by either agar plate culture or Baermann funnel technique [22]. Our results show that the diagnostic methods had a significant difference with the agar plate culture in diagnosing *S. stercoralis* infection. Sensitivity of the agar plate culture compared to the Baermann funnel technique, formalin-ether concentration, and direct smear examination was 1.3, 2, and 2.6 folds, respectively. The results of Baermann funnel technique and direct smear examination are in keeping with previous studies [22, 41].

We observed that 75% and 34.6% of the positive cases by the agar plate culture and ELISA test were patients treated by steroids, respectively. The most common risk factors for fatal hyperinfection or disseminated infection in immunocompromised patients are immunosuppression caused by corticosteroids, HTLV-1, and HIV infections [38]. Corticosteroids by interfere with the Th2 response via binding glucocorticoid receptors in the CD4 + Th2 cells may change the regular mechanisms of immunity. In addition, corticosteroids may also lead to dissemination through increased number of filariform larvae [7]. Glucocorticoids, the most widely used immunosuppressive drugs prescribed, are the most specifically associated with altering chronic strongyloidiasis to hyperinfection [28].

Of the eight positive cases by agar plate culture, we could only follow-up two cases. In one case, the agar plate culture was positive 6 months after treatment and in the other case it was positive 8 months after treatment. Considering that the test results of both patients were negative in the first and second visits after treatment, it seems that chronic cases of the disease are not easily detectable by the culture. Therefore, the importance of treating ELISA-positive patients with ivermectin, particularly in endemic areas is raised.

This study had limitations that should be considered. First, only one stool sample was examined from each patient. Second, ivermectin was not available in the region and patients were treated with albendazole. Third, due to the limited budget, PCR was performed only on culture-positive samples. Forth,

data on the CD4 and Viral load of the HIV patients, and the duration of treatment with corticosteroids was not available in all patients.

Conclusion

In conclusion, the majority of the positive cases by the agar plate culture had a history of prolonged exposure to soil, history of asthma and COPD, and were over the ages of 60. Thus, our findings stress the importance of screening for strongyloidiasis in high-risk patients, particularly in endemic areas. Given that the ELISA test had the highest sensitivity, screening of all high-risk patients for *S. stercoralis* infection is recommended prior to starting therapy using ELISA test. Considering the fact that in two cases *S. stercoralis* larva was observed 6 months and 8 months after treatment with albendazole, the monitoring of the patients is recommended. In addition, ivermectin should be available to the strongyloidiasis patients in the endemic areas.

Abbreviations

NTDs: neglected tropical diseases; COPD: chronic obstructive pulmonary disease; PCR: Polymerase chain reaction; STH: soil-transmitted helminthiasis; HTLV-1: human T-cell lymphotropic virus type 1; HIV: human immunodeficiency virus; DM: diabetes mellitus; ELISA: enzyme-linked immunosorbent assay; *SSU*: small subunit (18S) ribosomal DNA; ROC: Receiver Operating Characteristic; PPV: positive predictive value; PNV: negative predictive value; CBC: complete blood count; AUC: area under the curve

Declarations

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Ethics statement

The study protocol was reviewed and approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (Approval No IR.AJUMS.MEDICINE.REC.1398.039). All patients were informed of the

study aim and the procedures, and written informed consent was obtained prior to enrollment. For patients aged under 15 years old, written informed consent was obtained from their parents. All patients infected with *S. stercoralis* were treated with albendazole 400 mg/day orally for 3-7 days (the only drug currently available in the studied region).

Consent for publication

Not applicable.

Conflict of interests

The authors declare that they have no competing interest.

Authors' contributions

Conceived and designed the experiments: MB, AR. performed the experiments: AA. Analyzed the data: MB. Contributed reagents/materials/analysis tools: MB, AR, AA, AKh, AA. Wrote the paper: MB, AR.

Availability of data and materials

Not applicable.

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Figures

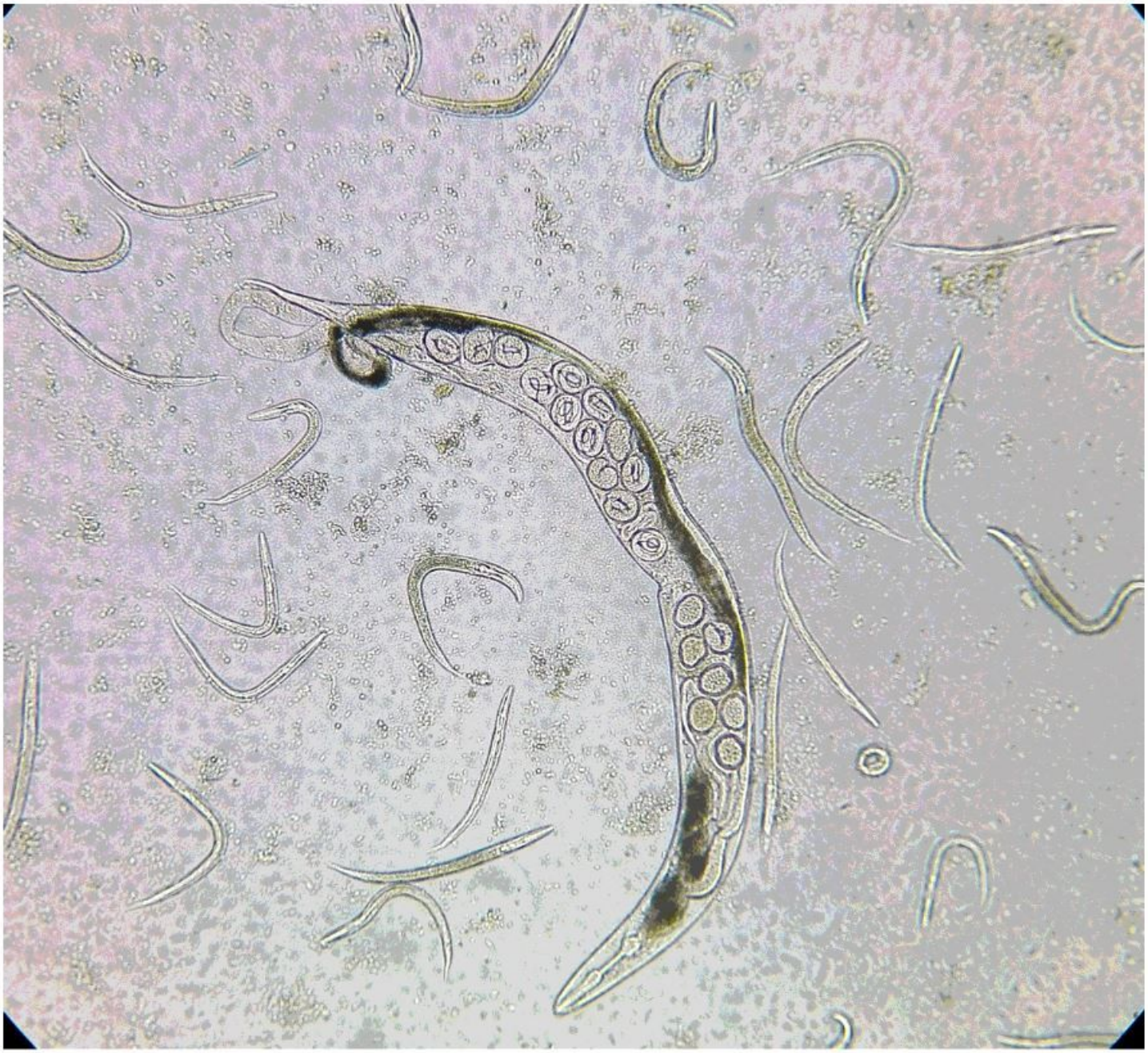

Figure 1

Adult female worm, rhabditiform larva, and ova collected from an agar plate culture of an infected patient studied in this study.

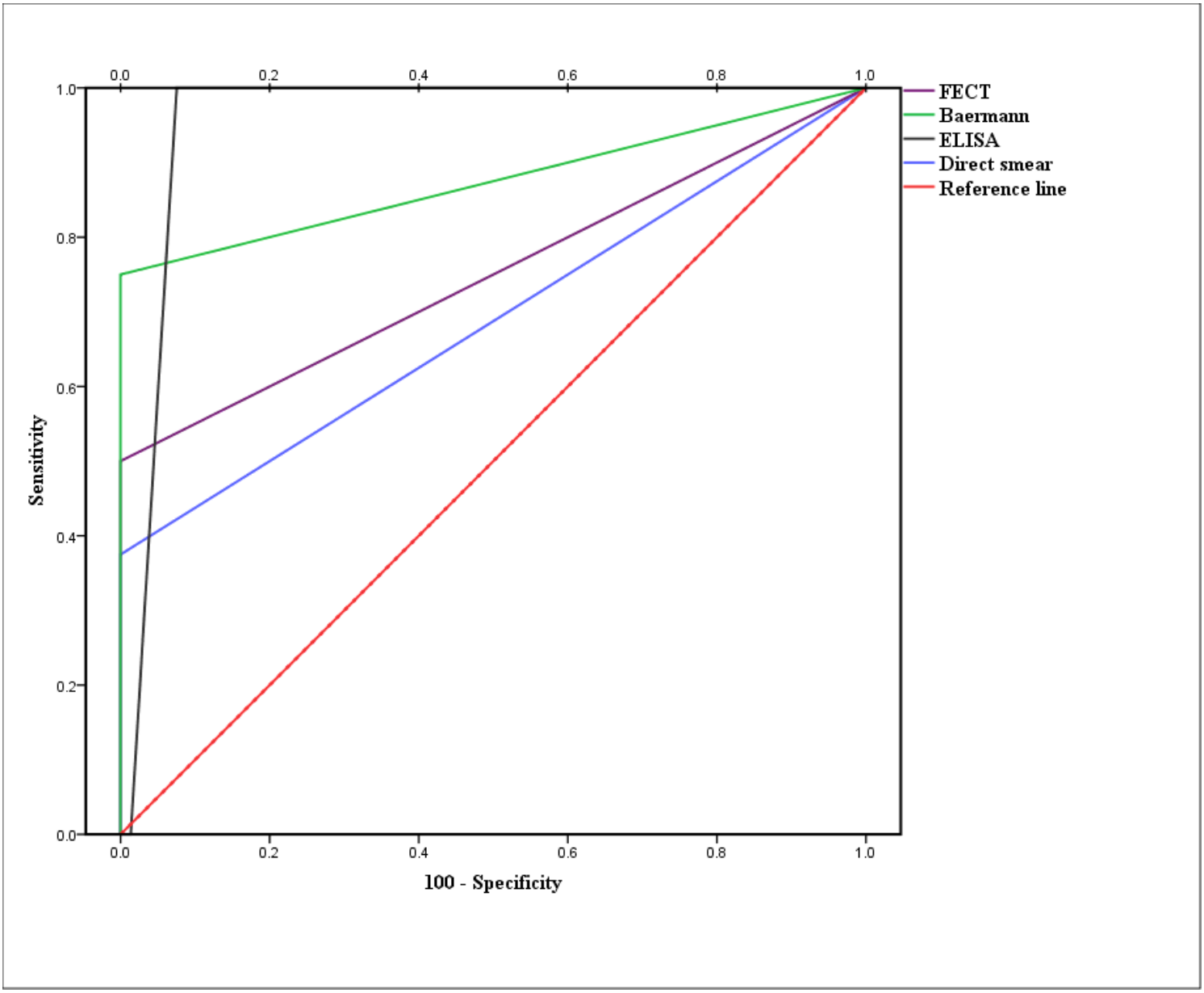

Figure 2

The ROC curves show the comparison between the agar plate culture and other tests used. FECT: Formalin-ether concentration technique. The ELISA Roc had an AUC (area under the curve) of 0.955 with 100% sensitivity, the Baermann funnel technique had an AUC of 0.875 with 70% sensitivity, the formalin-ether concentration Roc had an AUC of 0.750 with 50% sensitivity, and the direct smear examination ROC had an AUC of 0.688 with 37.5% sensitivity.

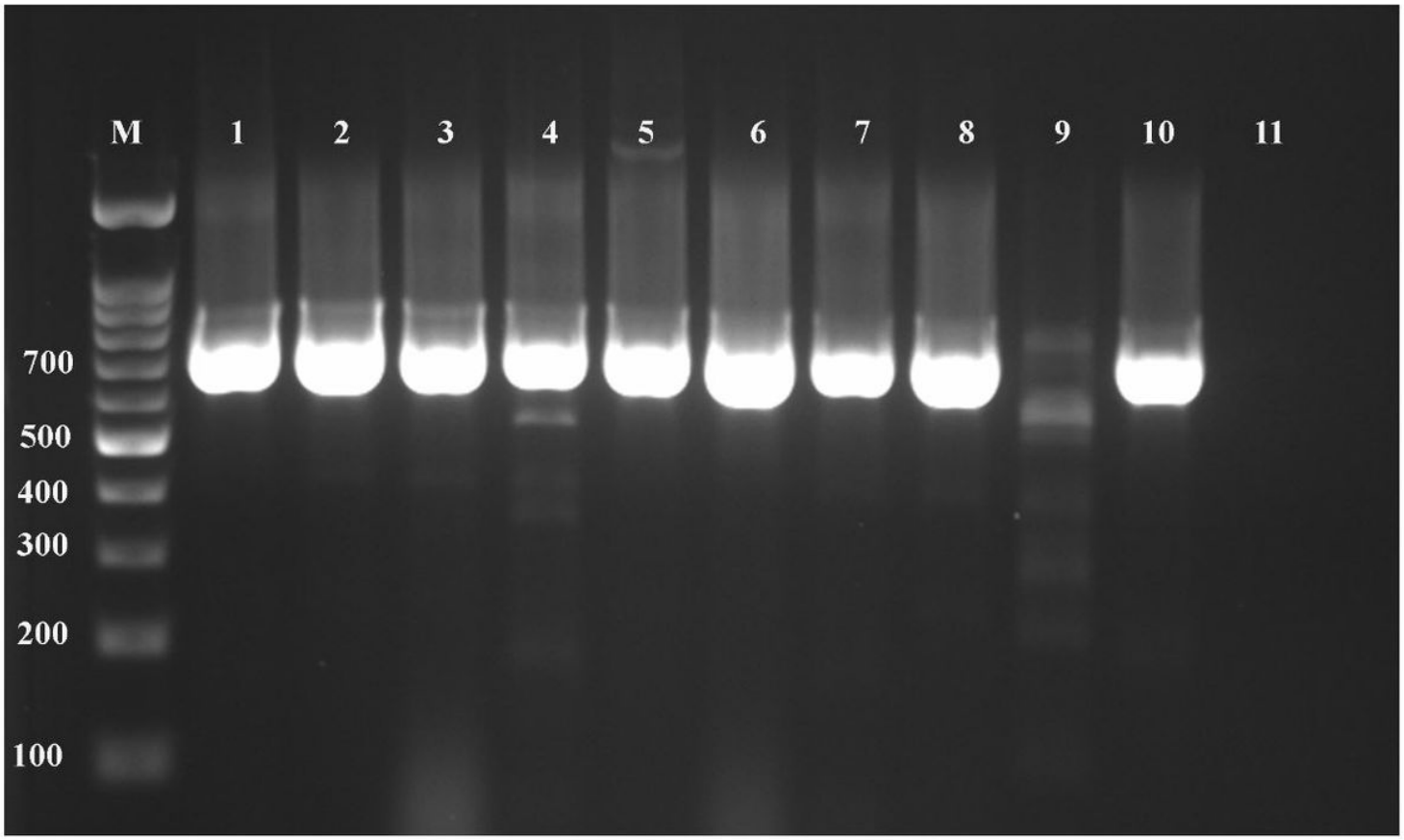

Figure 3

PCR amplification of a 712- bp fragment of the nuclear 18S rDNA (SSU) hypervariable region (HVRIV) of S. stercoralis from larva in feces of high-risk patients from Khuzestan Province, Iran. Lane M, 100 bp DNA ladder; Lane 1–8 positive samples, Lane 9–10 positive controls of S. stercoralis, Lane 11 negative control.