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Original Article

Sero-Parasitological Assessment of *Strongyloides stercoralis* among Solid Organ Transplantation Candidates at Imam Khomeini Hospital, Tehran, Iran

Rosesadat Shamsa [1], Zahra Ahmadinejad [2], Marjan Sohrabi [3], *Zohreh Fakhrieh-Kashan [4], Salman Zafari [4], *Fereshteh Ghiasvand [2]

1. *Department of Infectious Diseases, Sina Hospital, Tehran University of Medical Sciences, Tehran, Iran*
2. *Liver Transplant Research Center, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran*
3. *Department of Infectious Diseases, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran*
4. *Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*

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***Correspondence Emails:**
ghiasvandfereshte@gmail.com,
zfk579@gmail.com,
zfakhrieh@tums.ac.ir

Abstract

Background: *Strongyloides stercoralis* can cause hyperinfection or disseminated strongyloidiasis in solid organ transplant candidates receiving immunosuppressive drugs. We investigated the serological and parasitological characteristics of *S. stercoralis* in solid organ transplant candidates.

Methods: This cross-sectional study was conducted from March 2023 to March 2025 at Imam Khomeini Hospital Complex, Tehran, Iran. Of 252 patients initially assessed, 179 were included in the final analysis. Stool samples, available for a subset of patients due to collection and processing limitations, were examined using direct smear, formalin-ethyl acetate concentration, and agar plate culture. Serum samples were tested for anti-*S. stercoralis* antibodies using an indirect ELISA (NovaLisa®, recombinant NIE antigen). Associations were assessed using Chi-square or Fisher's exact tests.

Results: Seropositivity was detected in 12/179 patients (6.7%), whereas no *S. stercoralis* larvae were detected in 65 stool samples. The mean age was 50 years, and 59.5% were male. Seropositivity was significantly associated with residence or birth in northern Iran (P= 0.006), while lower educational attainment showed borderline significance (P= 0.050). No significant associations were observed with other demographic, clinical, or exposure-related variables.

Conclusion: ELISA identified seropositive cases despite negative parasitological findings, suggesting that routine stool examination alone may be insufficient for screening solid organ transplant candidates.

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Introduction

Transplantation of solid organs, e.g., kidneys, livers, hearts, and lungs are a life-saving treatment for patients with advanced organ failure. Despite significant advances in surgical technology, immunosuppressive drugs, and post-transplant care, mortality after transplantation remains a major challenge (1). Infections are among the most important causes of mortality after transplantation, which can occur due to the mandatory use of immunosuppressive drugs. These drugs weaken the patient's immune system and predispose to a variety of viral, bacterial, fungal, and parasitic infections (2). Recent research shows that infections are the leading cause of death in the first three months after transplantation and remain a major threat to patient survival in the long term. This highlights the need for novel approaches to prevent, early detection, and effectively treat infections in organ transplant recipients (3).

S. stercoralis is a soil-transmitted helminth (STH) that can live in the host for years without symptoms. Strongyloidiasis has an estimated global prevalence of around 8.1%, corresponding to 614 million infected people, particularly in developing countries located in South East Asia, the Middle East, Africa and Latin America, although precise data on prevalence are unknown in endemic countries (4, 5). In Iran, this parasitic infection is endemic in the northern regions, especially Guilan and Mazandaran provinces, and in the southern regions, including Khuzestan and Hormozgan provinces, where adequate temperature and humidity provide favorable conditions for parasite survival (6). In patients with a healthy immune system, the parasite is usually associated with mild gastrointestinal or skin manifestations; however, in immunocompromised patients, especially after solid organ transplantation it may become active and manifest in a severe and life-threatening form known as hyperinfection or disseminated strongyloidiasis syndrome (7). After organ transplantation, immunosuppressive drugs, e.g., corticosteroids, tacrolimus, or cyclosporine are prescribed to prevent rejection. These drugs reduce the cellular immune response and cause the previously dormant *Strongyloides* to proliferate and enter internal migratory cycles from the intestine to lung (8). As a result, the patient develops severe larval overgrowth in the intestine and lungs, extensive tissue damage, and concurrent opportunistic infections, leading to symptoms including gastrointestinal (e.g., diarrhea, abdominal pain, nausea, vomiting), pulmonary (e.g., cough, shortness of breath, hemoptysis), cutaneous (e.g., migratory rash, pruritic rash), and systemic manifestations (e.g., fever, sepsis, shock, impaired level of consciousness) (9, 10).

Different methods have been defined for the detection of *S. stercoralis*, including parasitological, immunological and molecular techniques. Although parasitological methods are the gold standard of diagnosis, they have limitations, e.g., low sensitivity and time-consuming (11, 12). Molecular methods, particularly PCR assays targeting ribosomal or mitochondrial genes of *S. stercoralis*, have been developed to overcome some limitations of conventional stool examinations. These assays generally show high specificity and, in several studies, higher sensitivity than direct microscopy and concentration techniques, especially in individuals with low or intermittent larval output. Real-time PCR and nested PCR formats have further improved analytical sensitivity and reduced contamination risk (13).

Immunological techniques, e.g., ELISA, have been used for the *S. stercoralis* detection and have certain advantages over parasitological methods (11). ELISAs using recombinant antigens that do not cross-react with other STHs have been developed to increase specificity and reduce turnaround time. In addition, these recombinant antigens may improve the

reproducibility, scalability, and sensitivity of serological assays (14-17).

Iran is considered an endemic region for *S. stercoralis*, particularly in the north and south of the country, and Imam Khomeini Hospital Complex is one of the high-volume organ transplant centers in the country and the Middle East, where more than a hundred and fifty liver transplants are performed annually.

We aimed to evaluate the seroprevalence and parasitological detection rate of *S. stercoralis* in solid organ transplant candidates to determine the most effective screening approach in Imam Khomeini Hospital Complex, Tehran, Iran.

Materials and Methods

Ethics statement

This study was approved by the Research Ethics Committee of the Tehran University of Medical Sciences, Tehran, Iran (Code No. IR.TUMS.IKHC.REC.1401.321) and informed consent was obtained from all patients before enrollment.

Study design

This cross-sectional study was conducted to investigate the accuracy of diagnostic methods of *S. stercoralis* in solid organ transplant candidates at Imam Khomeini Hospital complex from Mar 2023 to Mar 2025. Imam Khomeini Hospital Complex, affiliated with Tehran University of Medical Sciences, Tehran is known as the largest medical care, educational, and research center in Iran. This complex is a leader in solid organ transplant programs, e.g., liver, kidney, kidney- pancreas and heart, and its liver transplant program has been launched since 2001.

Participants in this study were selected from patients referring to transplant clinic for solid organ transplantation due to different etiologies (passive sample collection). The exclusion criteria included taking anthelmintic medication within the past 6 months, failure to attend for testing, persistently equivocal results in ELISA after repeat testing or failure to return for repeat sampling, and loss of serum during laboratory preparation.

Sample size and data collection

A data sheet was used for data gathering including demographic information (e.g., gender, age, education level, place of residence or birth), lifestyle (e.g., contact with animals, contact with soil), and type of transplant candidate organ. Clean plastic containers were provided to participants or their companions, and stool samples were collected next day. Moreover, two ml venous blood samples were obtained from each participant and their serum were stored at -20 °C until ELISA testing in Department of Infectious Diseases, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran. Totally, 179 patients were included in our study. Serum samples were obtained from all patients, but only 65 patients provided stool samples because of limited patient compliance and various clinical and logistical circumstances.

Parasitological tests

A single fresh stool specimen was obtained from each participant who provided a stool sample. Each specimen was examined using direct smear microscopy, formalin–ethyl acetate concentration, and agar plate culture for the detection of *S. stercoralis* larvae. For direct smear microscopy, one smear was prepared from each specimen and examined microscopically. The formalin–ethyl acetate concentration technique and agar plate culture were performed using standard procedures as previously described (18, 19). The agar plates were incubated at 25–30 °C for 7 d and inspected daily for larval tracks.

ELISA

The indirect ELISA was carried out utilizing the NovaLisa® Strongyloides antibody kit (NovaTec Immunodiagnostica GmbH, Dietzenbach, Germany) and the BioTek ELx808 analyzer (Winooski, Vermont, USA). This kit

identifies total IgG antibodies directed against *S. stercoralis* via an indirect ELISA approach that incorporates a recombinant immunodiagnostic antigen (NIE). The assay was executed following the manufacturer's guidelines, with outcomes expressed in NovaTech units. Values surpassing 11 NTU were interpreted as positive (and subjected to rechecking), those under 9 NTU were deemed negative, and readings ranging from 9 NTU to 11 NTU were classified as equivocal. Patients with initial equivocal results were invited for repeat sampling and ELISA testing after 4 wk.

Statistical analysis

The results were reported in the form of descriptive statistics as mean and number (%) for quantitative and qualitative variables, respectively. The Chi-square or Fisher's exact test was used to compare the distribution of individuals based on demographic and clinical characteristics, taking into account the serological results of the patients. The Python (SciPy library version 1.13.1) was used to analyze the data. A probability value (*P*-value) less than 0.05 was considered as a significant level.

Results

Antibodies against *S. stercoralis* were detected in 6.7% (12/179) of solid organ transplant candidates by indirect ELISA. In contrast, none of the 65 stool samples examined by parasitological methods (direct smear, formalin–ethyl acetate concentration, and agar plate culture) showed *S. stercoralis* larvae. Among the 65 participants who provided stool samples for parasitological examination, two were seropositive for *S. stercoralis*.

The mean age of the 179 patients included in the serological analysis was 50.01 years (SD 14.39), and 59.5% were male. Most patients had an educational level below a high school diploma (62.3%), and liver failure was the most common underlying condition (67%).

Positive serology was more prevalent in women (7.1%) than in men (6.4%), but this difference was not statistically significant (P= 0.851) (Table 1). Patients with positive serology had a lower literacy level than those with negative serology, and this difference was statistically significant (P= 0.050). Although a higher prevalence of *S. stercoralis* infection was observed in kidney transplant candidates (4/54; 7.4%) compared with liver transplant candidates (8/123; 6.5%), this difference was not statistically significant (P=0.789). In addition, having a residence or birthplace in northern Iran, an endemic area for *S. stercoralis*, was a prominent risk factor (23.8% vs. 4.4%; P= 0.006).

Following-up of patients showed that death occurred in four seropositive patients and was not statistically significant (P= 0.051). Clinical symptoms were observed in a limited number of positive cases (gastrointestinal, respiratory, and skin symptoms, one each), which was not statistically significant (P> 0.05). The significance level and other information of the characteristics examined in this study are shown in Table 1.

Initial ELISA testing identified nine patients with equivocal results (9–11 NTU). These patients were invited for repeat sampling and testing after 4 wk. Seven patients returned for follow-up; three tested positive and four tested negative. Two patients were excluded from the final analysis because they did not return for repeat sampling.

Table 1: Seroprevalence of *Strongyloides stercoralis* in solid organ transplant candidates of the Imam Khomeini Hospital Complex, Tehran, Iran

Characteristics		ELISA results		Total (%)	*P*-value
		Negative (%)	Positive (%)		
Gender	Female	65 (92.9)	5 (7.1)	70 (39.1)	0.851
	Male	102 (93.6)	7 (6.4)	109 (60.9)	
Age (yr)	<30	14 (93.3)	1 (6.7)	15 (8.4)	0.703
	30-39	30 (96.8)	1 (3.2)	31 (17.3)	
	40-49	38 (95.0)	2 (5.0)	40 (22.3)	
	50-59	30 (88.2)	4 (11.8)	34 (19.0)	
	>60	55 (93.2)	4 (6.8)	59 (33.0)	
Literacy	<Diploma	103 (94.5)	6 (5.5)	109 (60.9)	0.050
	Diploma	36 (85.7)	6 (14.3)	42 (23.5)	
	>Diploma	28 (100)	0 (0)	28 (15.6)	
Type of transplant candidate organ	Liver	115 (93.5)	8 (6.5)	123 (68.7)	0.789
	Kidney	50 (92.6)	4 (7.4)	54 (30.2)	
	Liver & kidney	1 (100)	0 (0)	1 (0.6)	
	Heart and kidney	1 (100)	0 (0)	1 (0.6)	
Cause of organ failure	Wilson's autoimmune hepatitis	0 (0)	1 (100)	1 (0.6)	0.507
	Non-alcoholic fatty liver	17 (85)	3 (15)	20 (11.2)	
	Cryptogenic	11 (91.7)	1 (8.3)	12 (6.7)	
	Metabolic syndrome	15 (93.8)	1 (6.2)	16 (8.9)	
	Other	124 (95.4)	6 (4.6)	130 (72.6)	
How to wash vegetables	Water	120 (91.6)	11 (8.4)	131 (73.2)	0.185
	Disinfectant	47 (97.9)	1 (2.1)	48 (26.8)	
Place of residence or birth	Northern Provinces	16 (76.2)	5 (23.8)	21 (11.7)	0.006
	Other Provinces	151 (95.6)	7 (4.4)	158 (88.3)	
Contact with soil	No	110 (90.9)	11 (9.1)	121 (67.6)	0.133
	Yes	57 (98.3)	1 (1.7)	58 (32.4)	
Contact with animals	No	134 (93.7)	9 (6.3)	143 (79.9)	0.956
	Yes	33 (91.7)	3 (8.3)	36 (20.1)	
Cortisone use	No	142 (92.8)	11 (7.2)	153 (85.5)	0.836
	Yes	25 (96.2)	1 (3.8)	26 (14.5)	
Patient death	No	148 (94.9)	8 (5.1)	156 (87.2)	0.051
	Yes	19 (82.6)	4 (17.4)	23 (12.8)	
Gastrointestinal symptoms	No	154 (93.3)	11 (6.7)	165 (92.2)	1.00
	Yes	13 (92.9)	1 (7.1)	14 (7.8)	
Respiratory symptoms	No	144 (92.9)	11 (7.1)	155 (86.6)	1.00
	Yes	23 (95.8)	1 (4.2)	24 (13.4)	
Skin symptoms	No	155 (93.4)	11 (6.6)	166 (92.7)	0.923
	Yes	12 (92.3)	1 (7.7)	13 (7.3)	

Discussion

In the present study, the serological prevalence of *S. stercoralis* based on ELISA in solid organ transplant candidates was 6.7% (12/179). This prevalence was compared with similar studies, e.g., Al-Obaidi et al. in Houston, USA, reported a prevalence of 9.9% in 1689 kidney transplant candidates (20); Telchik et al. observed a prevalence of 7.3% in kidney transplant candidates in Central Texas (21); and Tan et al. in the Philippines reported a higher prevalence of 25.8% (22). The observed differences are likely related to the level of endemicity of the geographical areas. Northern Iran is considered an endemic area for *S. stercoralis* (6), while Houston and Texas are non-endemic areas with limited migration, and the Philippines is a fully endemic area with higher environmental exposure. This

finding highlights the importance of considering organ transplant recipients' place of residence when assessing the risk of endemic infections in their region.

In our study, a significant association was observed between place of residence or birth in the northern provinces and seropositivity (P = 0.006), such that 23.8% of individuals living in these areas were positive compared to 4.4% in other provinces. This finding is consistent with the studies of Al-Obaidi and Telchik, who showed that geographical origin (such as Asian or Hispanic race and endemic areas) is the strongest predictor of seropositivity (20, 21). Greater exposure of parasite larvae to moist soil and suitable climatic conditions in northern Iran and native areas could be the reasons for this similarity. Therefore, the history of residence can be used as an important marker in risk assessment.

The level of literacy has a borderline significant association with seropositivity (P=0.050). This variable has been less studied in most previous studies, but could be related to personal hygiene practices (e.g., how to wash vegetables with plain water versus disinfectant). The indirect association of literacy level with hygiene behaviors is a plausible explanation for this difference and emphasizes the importance of hygiene education in reducing risk.

Contact with soil in this study did not show a significant association (P=0.133), although the prevalence was slightly higher in non-contact individuals. This finding is somewhat different from the study conducted in the Philippines (22), which identified barefoot walking as a strong independent risk factor (P=0.003). This difference may be related to the more precise definition of exposure behaviors (e.g., barefoot walking versus general soil contact) and sample size. However, both studies emphasize the need for behavioral modification, such as wearing appropriate footwear.

In the present study, no significant differences in prevalence were observed by sex (P=0.851), age groups (P=0.703) and type of transplanted organ (P=0.789). These results are fully consistent with the findings of Al-Obaidi et al. who reported that sex and age were not strong predictors (20). Therefore, the often silent nature of *Strongyloides* infection is less influenced by baseline demographic variables, and these factors alone are not sufficient for targeted screening.

Clinical features in this study, i.e., gastrointestinal, respiratory, and skin symptoms did not show a statistically significant association with positive serology. This result is consistent with data from many transplant studies (21, 23), as a large proportion of *Strongyloides* patients may be asymptomatic or have nonspecific symptoms in the pretransplant phase or even with positive serology. Serological tests are more indicative of previous exposure than necessarily of active symptomatic infection, and on the other hand, the severity of symptoms depends on the parasite load, immune status, and time of examination. In clinical practice, we may interpret a positive IgG ELISA in the context of epidemiological risk; in high-risk transplant candidates this may justify pre-emptive ivermectin treatment, whereas in low-risk individuals repeat testing or close follow-up may be considered before initiating therapy. Accordingly, serological screening be performed extensively in solid organ transplant candidates in endemic areas, to prevent severe infection and life-threatening complications.

None of the 65 stool samples examined in this study showed rhabditoid *S. stercoralis* larvae by three parasitological methods, i.e., direct smear, formalin-ethyl acetate, and agar plate culture. This finding is consistent with the insensitive nature of many routine stool methods for *S. stercoralis*, especially when the parasite load is low or the larvae are excreted intermittently (24). In contrast, more focused methods, e.g., agar plate culture and also combinations of diagnostic methods usually perform better than direct smear, but even

they are not always positive. Therefore, a negative parasitological test does not necessarily rule out previous infection and probably reflects the inherent limitations of stool methods in screening patients before transplantation. Although recombinant NIE-based ELISA assays have high diagnostic performance and lower cross-reactivity than crude-antigen assays, cross-reactivity with other helminth infections and persistent antibodies after previous infection cannot be completely excluded (25). In our study, intestinal parasitic co-infections could not be comprehensively excluded because only 65 stool specimens were available and a single specimen was examined per patient. Therefore, the absence of stool-positive cases does not rule out active *S. stercoralis* infection.

The limitations of our study included: (a) the exclusion of equivocal ELISA results, (b) the lack of a molecular gold standard (PCR) to confirm true positives, (c) the cross-sectional design preventing differentiation between active infection and past exposure, and (d) the potential impact of examining only a single stool sample on the low parasitological yield.

Conclusion

The seroprevalence of *S. stercoralis* among solid-organ transplant candidates was 6.7%, whereas no positive cases were detected using three stool-based parasitological methods. These negative parasitological results may be attributed to factors e.g., the limited number of available stool samples and the collection of only a single sample per patient, as well as the known limitations of stool testing in detecting *S. stercoralis* infections characterized by low parasite loads or intermittent shedding. These findings suggest that serological methods may offer added diagnostic value for pre-transplant screening compared to conventional parasitological techniques, particularly when stool sampling is limited.

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Conflict of interest

The authors declare that there is no conflict of interests.

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