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Seroprevalence of *Strongyloides stercoralis* among patients with leptospirosis in northern Iran: a descriptive cross-sectional study

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Abstract

Strongyloidiasis and leptospirosis are neglected tropical diseases that have affected many countries across the world. In this study, we evaluated the seroprevalence of *Strongyloides stercoralis* among patients with leptospirosis in northern Iran. We evaluated 156 laboratory- or clinically confirmed leptospirosis cases. The overall seroprevalence of *S. stercoralis* was 32.0% (95% confidence interval (CI): 27.4–36.5%, 50/156). Seropositivity was significantly associated with agricultural activities (odds ratio (OR): 2.84, 95% CI: 1.0–8.77) and gastrointestinal disorders (OR: 2.4, 95% CI: 1.1–4.9). Laboratory findings indicated that seropositivity of *S. stercoralis* was significantly associated with decreased levels of platelet and elevated levels of creatinine, alanine aminotransferase and aspartate aminotransferase ($P < 0.05$). Our findings suggested a higher exposure to *S. stercoralis* larvae among patients with leptospirosis. The public health and medical communities may benefit from this research through preventive measures to improve farmers' knowledge and awareness regarding strongyloidiasis and leptospirosis and the associated risk factors.

Introduction

Leptospirosis, caused by gram-negative bacteria belonging to the genus *Leptospira*, is an emerging zoonotic disease that has affected many countries worldwide (Palaniappan *et al*., 2007; Tilahun *et al*., 2013; Sohail *et al*., 2018). Annual worldwide morbidity and mortality of leptospirosis are estimated to be almost 1.03 million and 58,900 cases, respectively (Costa *et al*., 2015). Humans are infected when the excreted leptospires by infected animals in water or soil is entered the body through impaired skin barrier or mucous membranes (Hartskeerl *et al*., 2011). The spectrum of leptospirosis is broad; infected people can be asymptomatic or present self-limited acute febrile illness, while severe infection can lead to myocardial infection, hepatic disease, kidney failure and pulmonary haemorrhage syndrome. The complications are responsible for the death of 5–15% of hospitalized patients (Bharti *et al*., 2003). It is hypothesized that leptospirosis severity may be increased in coinfection with other infectious agents (Bharti *et al*., 2003). One of these agents is *Strongyloides stercoralis*, which is co-endemic with *Leptospira* spp. and has a similar transmission route and targeted organs.

Strongyloides stercoralis is the cause of a neglected helminthic disease known as strongyloidiasis with a worldwide distribution, particularly in developing countries located in South East Asia, the Middle East, Africa and Latin America (Schär *et al*., 2013). Due to an autoinfective life cycle, strongyloidiasis can persist in the host for several decades (Grove, 1996)). It is estimated that almost 370 million people around the world have been affected by strongyloidiasis (Schär *et al*., 2013; Krolewiecki & Nutman, 2019). The majority of infections are either asymptomatic or are mild symptoms such as gastrointestinal disorders, dermatitis on the skin and lesions in the lungs (Krolewiecki & Nutman, 2019). However, the infections can lead to serious damages in immunocompromised people such as those with human immunodeficiency virus (HIV)/AIDS or human T-cell lymphotropic virus-1 (HTLV-1), patients taking corticosteroids or immunosuppressive drugs like cancer or organ transplant patients (Buonfrate *et al*., 2013; Asundi *et al*., 2019). Several coprological assays have been used to diagnose *S. stercoralis*, including direct stool smears, Kato-Katz, MiniFLOTAC, the Baermann technique, charcoal cultures, the Harada–Mori filter paper culture and nutrient agar plate cultures (Ericsson *et al*., 2001; Buonfrate *et al*., 2015a). However, these assays have been in-process laborious with poor sensitivity (Buonfrate *et al*., 2018a; Krolewiecki & Nutman, 2019). Moreover, molecular techniques such as real-time polymerase chain reaction have not shown high sensitivity (Buonfrate *et al.*, 2018b). Serological assays such as enzyme-linked immunosorbent assay (ELISA) have been used in a growing body of literature in recent years with a higher sensitivity than stool examinations. However, based on test performance and antigens used, the sensitivity of these serological assays varies from 71% to 95%. The main limitation for serological tests is the overestimation of prevalence as a result of false-positive tests due to cross-reactivity with other helminths (Requena-Méndez *et al.*, 2013; Buonfrate *et al.*, 2015b; Asundi *et al.*, 2019).

Northern regions of Iran are co-endemic for leptospirosis with many parasitic diseases, including strongyloidiasis (Abdollahpour *et al.*, 2009; Ashrafi *et al.*, 2010; Yakhchali *et al.*, 2011; Ahmadi *et al.*, 2015; Ghasemian *et al.*, 2016). This co-endemicity may lead to difficulties in diagnosis and early treatments in exposed patients. Thus, the main aim of this study is to estimate the seroprevalence of *S. stercoralis* among patients with leptospirosis in northern Iran and to evaluate the associated clinical symptoms and laboratory findings.

Methods

All the procedures of this descriptive hospital-based cross-sectional study were approved by the Ethics Committee of the Babol University of Medical Science, Mazandaran, Iran (no. IR. MUBABOL. HRI. REC. 1398. 368). The Mazandaran province is located along the southern coast of the Caspian Sea in northern Iran. The main economic activity of people in this province is agriculture, and most people are occupied in rice production. Samples were collected from May 2018 to January 2020 in Rouhani Hospital, Babol, one of the leading hospitals in northern Iran. We used a simple random sampling technique. The patients infected with HIV, patients with autoimmune disease and patients who used immunosuppressive drugs were excluded from this study. The single venous samples (5 ml) were collected from all participants and then transported to the Pasture Institute, Amol, Mazandaran, and centrifuged at 1000 g for 10 min. Finally, the separated sera were stored at −20°C. All suspected individuals were screened for the presence of IgG and IgM antibodies to *Leptospira* using a commercially available ELISA kit (PanBio, Baltimore, USA). Moreover, the suspected individuals were clinically evaluated by an infectious disease specialist.

In total, 156 clinical or laboratory-confirmed patients for leptospirosis were included in this study. First, the socio-demographic, clinical features and laboratory information of all participants were collected from the hospital records. Then, the sera samples for all confirmed patients were tested for anti-*Strongyloides* IgG antibodies using a commercial ELISA kit (NovaTec Immunodiagnostics, Dietzenbach, Germany) following the manufacturer's instructions. This kit has reported clinical sensitivity and specificity of >95%. To avoid bias, all serological tests were conducted by a trained laboratory technician who was unaware of the status of the study subjects. According to the manufacturer's instructions, sera with international units (IU)/ml of <9.0, 9.0–11.0 and >11 IU/ml were considered as negative, suspicious and positive subjects, respectively.

Data were entered and analysed by SPSS Statistics software, version 21 (IBM, Armonk, New York, USA). Descriptive data for both groups were presented using the relative frequency with an exact binomial at 95% confidence intervals (CIs). Independent *t*-tests were used to evaluate the correlation between two normally distributed quantitative data. In addition, a Chi-square test was used to assess the correlation between qualitative data. A *P*-value of <0.05 was accepted as a statistically significant association.

Results

In this study, we included 156 patients, including 90 laboratory and 66 clinically confirmed cases of leptospirosis. The median age of patients was 49.3 ± 14, and the majority of cases were males (87.2%, *N* = 136). Serological assessment of the patients showed that 50 leptospirosis patients (32.0%, 95% CI: 27.4–36.5%) were positive for anti-*S. stercoralis* IgG antibodies. Moreover, 33 subject (36.6%) of laboratory confirmed patients, and 17 subjects (25.7%) of clinically confirmed patients had anti-*S. stercoralis* IgG antibodies, respectively. Based on socio-demographic characteristics, agricultural activity was marginally associated with seropositivity to *S. stercoralis* (odds ratio (OR): 2.84, 95% CI: 0.98–8.77, *P*-value = 0.05). Moreover, the patients who travelled to neighbouring provinces for work in rice fields (14.8%, 95% CI: 9.7–19.8%) had significantly lower (OR: 0.31, 95% CI, 0.1–0.3; *P*-value = 0.03) seroprevalence than patients who worked only in Mazandaran province (35.6%, 95% CI, 30.0–41.1%) (table 1).

Clinical characteristics of patients indicated that seropositivity to *S. stercoralis* was associated with gastrointestinal disorders (OR: 2.4, 95% CI: 1.1–4.9) (supplementary table 1). Laboratory results suggested that seropositivity for *S. stercoralis* was significantly associated with increased levels of the platelet (OR: 2.6, 95% CI: 1.1–5.8), creatinine (OR: 2.6, 95% CI: 1.0–7.4), alanine aminotransferase (OR: 2.4, 95% CI: 1.0–6.2) and aspartate aminotransferase (OR: 3.0, 95% CI: 1.1–8.3) at 95% confidence level. Detailed socio-demographic and laboratory characteristics of *S. stercoralis* serostatus are presented in table 1.

Discussion

To the best of our knowledge, this is the first study that evaluated the co-infection of strongyloidiasis and leptospirosis. Our results indicated that approximately one-third (32%) of patients with leptospirosis are seropositive for strongyloidiasis. This seroprevalence rate is slightly higher than previous studies in the north of Iran among the high-risk population. Previous studies reported the seroprevalence rates of strongyloidiasis as 25.6%, 27.9% and 30% in diabetic patients, suspected patients to infectious diseases and immunocompromised patients, respectively (Gorgani-Firouzjaee *et al.*, 2018; Javanian *et al.*, 2019; Kalantari *et al.*, 2019). While the seroprevalence rate reported in our study is much higher than previous reports in Khuzestan Province, south-west of Iran, where the seroprevalence rates were 8.7% and 14.4% among high-risk patients (Rafiei *et al.*, 2016; Ashiri *et al.*, 2021). A possible explanation for the high seroprevalence rate in patients with leptospirosis could be due to similar transmission routes of both infections among people/farmers working on rice fields who are at higher risk of infections. Another hypothesis for this high rate would be cross-reaction with other helminths (*Ascaris lumbericoides* or *Toxocara* spp.) and maybe *Leptospira* antibodies; however, there is no strong evidence for this argument (Mohammadzadeh *et al.*, 2020; Darvish *et al.*, 2021).

With respect to risk factors, our findings indicated that the seroprevalence of strongyloidiasis was higher in males, and patients associated with agricultural activities. It should be

Table 1. Seroprevalence of *Strongyloides stercoralis* in patients with leptospirosis based on socio-demographics and laboratory findings.

Variables	Seropositive patients for *S. stercoralis* *n* (%)	Seronegative patients for *S. stercoralis* *n* (%)	Odds ratio (95% CI)	*P*-value
Sex				0.46
Male	45 (90.0)	91 (85.8)	1.48 (0.5–4.33)	
Female	5 (10.0)	15 (14.2)	1	
Age				
≤40	10 (20.0)	30 (28.3)	1	
41–60	26 (52.0)	57 (53.7)	1.36 (0.8–2.1)	0.47
>60	14 (28.0)	19 (18.0)	2.21 (0.6–3.5)	0.11
Residence				0.28
Urban	17 (34.0)	39 (36.8)	0.88 (0.43–1.79)	
Rural	33 (66.0)	67 (63.2)	1	
Agricultural activity				0.05
Yes	46 (92.0)	85 (80.2)	2.84 (1.0–8.77)	
No	4 (8.0)	21 (19.8)	1	
Contact with animal				0.34
Yes	11 (22.0)	31 (29.2)	0.68 (0.31–1.50)	
No	39 (78.0)	75 (70.8)	1	
Contact with stagnant water				0.27
Yes	31 (62.0)	75 (70.8)	0.67 (0.33–1.36)	
No	19 (38.0)	31 (29.2)	1	
Trip in last six months				0.03
Yes	4 (8.0)	23 (21.7)	0.31 (0.10–0.96)	
No	46 (92.0)	83 (78.3)	1	
White blood cell (WBCs)				0.2
>10,000	25 (50.0)	64 (60.4)	0.6 (0.3–1.2)	
≤10,000	25 (50.0)	42 (39.6)	1	
Platelets				0.01
>150,000	40 (80.0)	64 (60.4)	2.6 (1.1–5.8)	
≤150,000	10 (20.0)	42 (39.6)	1	
Creatinine				0.05
>1.5	9 (18.0)	8 (7.5)	2.6 (1.0–7.4)	
≤1.5	41 (82.0)	98 (92.5)	1	
Bilirubin T2				0.3
>2	5 (10)	6 (5.7)	1.8 (0.5–6.3)	
≤2	45 (90)	100 (94.3)	1	
Bilirubin D2				0.4
>20%	34 (78.0)	65 (61.3)	1.3 (0.6–2.7)	
≤20%	16 (32.0)	41 (38.7)	1	
Alanine aminotransferase (ALT)				0.05
>two-fold	10 (20.0)	10 (9.4)	2.4 (1.0–6.2)	
≤two-fold	40 (80.0)	96 (90.6)	1	
Aspartate aminotransferase (AST)				0.02

(*Continued*)

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Table 1. (*Continued.*)

Variables	Seropositive patients for *S. stercoralis* *n* (%)	Seronegative patients for *S. stercoralis* *n* (%)	Odds ratio (95% CI)	*P*-value
>two-fold	10 (20.0)	8 (7.5)	3.0 (1.1–8.3)	
≤two-fold	40 (80.0)	98 (92.5)	1	

CI, confidence interval.

noted that these results should be interpreted with caution because of the small sample size of women tested in the present study. Also, based on statistical analysis, agricultural activities were marginally associated with higher seropositivity of *Strongyloides*. The higher seroprevalence of strongyloidiasis in male patients is consistent with previous studies in Thailand (Nontasut *et al*., 2005), Cambodia (Khieu *et al*., 2014) and China (Steinmann *et al*., 2007). This might be due to the male activities in muddy rice fields without footwear and more exposure to *Strongyloides* larvae.

Considering clinical symptoms, our findings suggested that seropositivity to *Strongyloides* was significantly associated with gastrointestinal disorders. Although approximately 50% of cases infected with *Strongyloides* are asymptomatic, previous studies reported that gastrointestinal disorders such as nausea and diarrhoea were frequently found in individuals infected with *Strongyloides* (Schär *et al*., 2013; Khieu *et al*., 2014). Other frequent symptoms were cutaneous signs such as itchiness and urticaria. In our study, as the clinical symptoms were retrieved from medical records, we were unable to accurately assess the itchiness and urticarial symptoms.

Based on the laboratory findings, our results showed that seropositive patients for *Strongyloides* had significantly higher levels of platelets, creatinine, alanine aminotransferase and aspartate aminotransferase than seronegative patients. These increasing levels, especially creatinine, could have clinical implications, as the rising level of creatinine is a potential risk factor of adverse outcomes in leptospirosis and is also considered as a predictor of lethality in severe leptospirosis (Spichler *et al*., 2008).

There are some limitations in this study that should be acknowledged, and, thus, the results should be interpreted with caution. First, the strongyloidiasis in our studied subjects was not evaluated using other confirmative parasitological methods such as stool examination or Western Blot. Moreover, because of the nature of serological methods, we were unable to separate the latent or acute phase of strongyloidiasis in our studied patients and we could not ignore the false-positive cases due to cross-reactivity with other parasites (*Toxocara* spp. or *Ascaris lumbricoides*). Moreover, we were unable to collect information regarding blood eosinophils and skin abnormalities because of incomplete medical records for recruited patients. Therefore, due to these limitations and also possible false-positive results, the differentiation of underlying infections that might mimic strongyloidiasis was not possible. For further studies, we suggest an in-depth assessment of the laboratory parameters and clinical symptoms of *S. stercoralis* infection in patients with leptospirosis.

In summary, despite the abovementioned limitations, this study showed that exposure to *Strongyloides* larvae is higher among patients with leptospirosis. Our results suggested that people working on muddy rice fields in Mazandaran province are at higher risk of both strongyloidiasis and leptospirosis. A learning health program is needed to increase farmers' knowledge regarding both strongyloidiasis and leptospirosis, their related risk factors and preventive measures. Moreover, a routine screen of high-risk people is suggested for early diagnosis and treatment. We also suggest more studies to evaluate the co-infection of strongyloidiasis and leptospirosis, especially in endemic areas such as South East Asia, Africa and South America.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S0022149X21000237.

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Conflicts of interest. None.

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