Is the Baermann, an applicable method for detection of *Strongyloides stercoralis*?

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Original Article

Abstract

Introduction: It is estimated that 100 - 300 million people in the world are infected with *Strongyloides stercoralis.* Although there are several laboratory diagnostic methods for detection of this parasite, there is still no gold standard diagnostic method. The need for an application test with high sensitivity and specificity is highly felt, especially in people with immune deficiency or organ transplant volunteers.

Methods: The diagnostic sensitivity of five parasitological methods, including direct smear of feces in saline and Lugol iodine stain, Baermann technique, formalin-ethyl acetate concentration, Harada-Mori filter paper, agar plate culture and molecular method was evaluated by stool specimens collected from the central mentally retardation institute of Bandar Abbas, to find out an easy, practical and cost-effective method for detecting *Strongyloides stercoralis.*

Results: Five parasitological and molecular methods were investigated using 163 stool samples. Considering the employed methods, there found to be 30 cases of *S. stercoralis*. The total positive cases of *S. stercoralis* using different methods was considered as golden standard. The highest sensitivity of parasitological methods was associated with the Baermann 27 (90%) and the least positive results obtained with Harada-Mori method, 1 (3.3%). In this study 9 positive cases were detected by PCR method (30%).

Conclusion: Highest sensitivity was observed when different diagnostic approaches were combined where Baermann technique proceeded it. Therefore, this technique is recommended as a selective method for detecting *S. stercoralis* due to its easy, practical and inexpensive nature.

Key words: Methods, Strongyloides Stercoralis, Diagnosis

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Introduction:

It is estimated that 100 - 300 million people in the world are infected with Strongyloides stercoralis (1). Identifying this parasite is important especially in the elderly people and those with immunosuppressed system (2, 3).

Since, S. stercoralis has the capacity of auto-infection, it can cause systemic infection with high parasite densities, especially in immunocompromised individuals (2). Detection of S. stercoralis is possible by observing the larvae of the parasite in the feces (4). It is often delayed to detect this parasite because the load of larvae is low or variable which results in sensitivity decrease of parasitological tests (5). Although there are several laboratory diagnostic methods

for the detection of this parasite, there is still no gold standard diagnostic method (2, 5). It is not possible to estimate accurately the prevalence of this parasite, because the sensitivity of the various methods is remarkably different, so that even with a three-stool exam, the results of direct method increases significantly (2). The need for an applicable test with high sensitivity and specificity is highly felt, especially in people with immune deficiency or organ transplant volunteers (6). New methods, such as serology, are also used but these tests possess weak points as ineffectiveness in detecting disseminated infections, cross-reactivity with other intestinal helminthes, as well as a slowly reduction in anti-body titer after treatment of patients (6, 7).

Concentration methods, such as Baermann technique and agar plate culture are efficient methods, but are not sufficient for the detection of the parasite (8). A meta-analysis study showed more acceptable results with the two methods of Baermann technique and agar plate culture (9).

However, these techniques cannot be standardized because they are not carried out uniformly throughout the world since the variation of amount of stool and incubation time may occur (10). The need to investigate different parasitological and molecular methods for the diagnosis and enhancement of its sensitivity and specificity is fully felt as well (6).

In this study, we used five parasitologicl methods, including direct smear of feces in saline and Lugol iodine stain, Baermann technique, formalin-ethyl acetate concentration, Harada-Mori filter paper and agar plate culture and finally molecular method, to find out an easy, practical and cost-effective method for detecting S. stercoralis.

Methods:

In order to conduct this descriptive analysis study, 163 stool specimens were collected from the central mentally retardation institute of Bandar Abbas during a year, 2016 - 2017. Bandar Abbas (27°11′N 56°16′E), located in Hormozgan province, in south of Iran (https://en. wikipedia. org/ wiki/ BandarAbbas).

The study was approved by the ethics committee (HUMS REC 1395.54). All relevant authorities and headmasters of the rehabilitation center were informed about the purpose and procedures of the study. Written informed consent was obtained from the parents or the legal guardians of the patients prior to study initiation.

A Chi-squre test was used to assess association between the categorical variables. Statistical analysis of the data was analyzed using SPSS (version 20, Chicago, IL, USA) software and $P<0.05$ was considered as a significant.

Field and laboratory procedures

After coordination with authorities of the center, the collected samples within 3 hours were transported to the parasitological laboratory of the faculty medicine, HUMS. A number of parasitic techniques and also molecular technique for detection of larvae of S. stercoralis were used including direct smear of feces in saline and Lugol iodine stain, Baermann technique, formalin-ethyl acetate concentration, Harada-Mori filter paper and agar plate culture (11).

Direct smear

For the detection of cyst and trophozoites of protozoa or helminthes' eggs and larva, the wet smear was prepared by saline and lugol, examined under a light microscope. For the best results, three stool samples were taken from each individuals.

Formalin-ethyl acetate concentration

As in formalin-ethyl acetate concentration method, the preserved fecal suspension was filtered through two layers of wet gauze into a centrifuge tube. The volume was adjusted to 10 ml with 10% formalin. Two milliliters of ethyl acetate were added, and the tube was closed and shaken. Then the tube was centrifuged at 600×**g** for 10 min. The plug of debris was discarded by inverting the tube, leaving only the sediment, which was suspended. The entire suspension was examined under a microscope (11).

Agar plate culture

For the detection of S. stercoralis larva, the nutrient agar plate culture was prepared. For this purpose, approximately 3 grams of stool sample was placed in the middle of the agar plate and incubated in room temperature. Following incubation, the plates were examined for the presence of S. stercoralis larvae under a light microscope. However, distinctive characteristics

can only be determined under a microscope by the short buccal cavity and large genital primordium of rhabditiform (L_1) for S. stercoralis larvae. The filariform larvae (L_3) of S. stercoralis can be identified by their characteristically forked tail (10).

Baermann method

As in the Baermann method, some pieces of the feces approximately a hazelnut-sized was placed on gauze inserted into a glass funnel filled with warm water. After about 12 hours the collected water was centrifuged and the sediment was evaluated by a microscope for the presence of S. stercoralis larvae (2).

Harada-Mori filter paper

The Harada-Mori technique, which is a filter-paper culture method, utilizes the water tropism of *Strongyloides* larvae to concentrate them. Briefly, fresh feces are deposited on filter paper which is soaked with water and then incubated for 10 days at 30°C. The water sediment is screened daily to look for living larvae (6).

Molecular detection

DNA extraction and PCR amplification

DNA was extracted from stool using QIAamp® DNA stool MiniKit (QIAGEN, Hilden, Germany) according to the manufacturer's manual. The DNA pellet was dissolved in 50µl of sterile distilled water, incubated in a water bath at 65°C for 5min, and stored at -20°C until use. Parasite species--specific primers, the internal transcribed spacer 1 and 5.8S ribosomal RNA was amplified by forward: 5´ (GTG GAT CAT TCG GTT CAT AG) 3´and reverse: 5´ (TA CTA CTT GTT ATC ACC CTCA) 3´primer pairs to obtain a specific 153bp product (12). PCR was performed by means of 2× PCR Master Mix Red – MgCl2:1/5 Mm amplicon (Iranian Novin Gen) in 30 cycles under the following condition: 95C° for 3 min, followed by 35 cycles of denaturation at 95°C for 20s, annealing 54°C for 30s, extension at 72°C for 30s, with a final extension step at 72°C for 5min. To confirm the optimization process, DNA samples extracted from filariform larvae were used as positive and distilled water alone were used as negative controls. PCR products were analyzed on 2% agarose gel.

Results:

Five parasitological and molecular methods were investigated using 163 stool samples. Considering all the employed methods, there found to be 30 (18.4%) cases of *S. stercoralis*. Given the positive cases of *S. stercoralis* using different methods as golden standard, the sensitivity of the used methods was shown in Table 1. The highest sensitivity of parasitological methods was associated with the Baermann method 27 (90%) and direct smear 16 (53.3%) respectively, however, the least positive results obtained with Harada-Mori method, 1 (3.3%). In this study 9 positive cases were detected by PCR method (30%). Rabditiform and filariform larvae of S. stercoralis and free-female of S. stercoralis were shown in Figures 1-3.

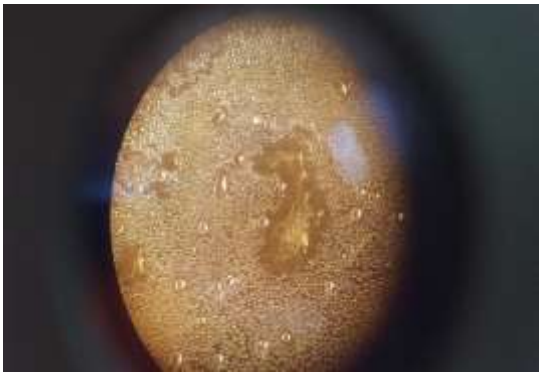

Figure 1: Agar plate culture, filariform larvae of Strongyloides stercoralis on plate lid.

Figure 2. Rabditiform larvae of Strongyloides stercoralis on direct smear

Table 1. Comparison of Baermann method with agar plate culture, direct smear of feces, formalin-ethyl acetate concentration, Harada-Mori filter paper, and PCR for detection of *S. stercoralis*

		Baermann			
		Positive	Negative	Total	Sensitivity (%)
Direct smear	Positive	16	0	16	59.3
	Negative	11	136	147	
	Total	27	136	163	
Formalin-ethyl acetate	Positive	8	3	11	29.6
	Negative	19	133	152	
	Total	27	136	163	
PCR	Positive	6	3	9	22.2
	Negative	21	133	154	
	Total	27	136	163	
Agar plate	Positive	5	0	5	16.7
	Negative	22	136	158	
	Total	27	136	163	
Marada-Mori	Positive	1	0	1	3.3
	Negative	26	136	162	
	Total	27	136	163	

* Comparison of five parasitological and molecular methods with Baermann to detect *S. stercoralis* larvae was statistically significant (P = 0.0001). The sensitivity of Baermann, calculated by combining results of all 5 methods, was 90% (27.30) for *S. stercoralis*.

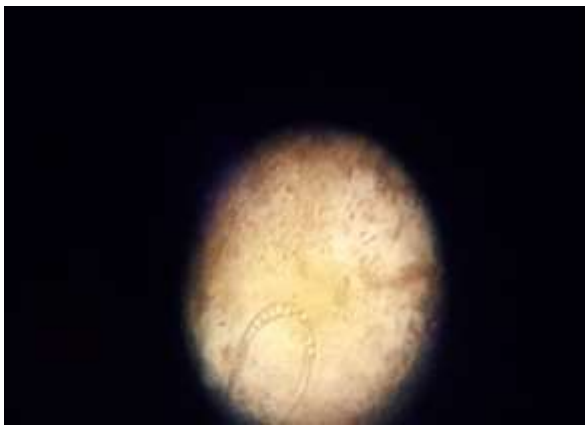

Figure 3. Free- female of Strongyloides stercoralis (Containing eggs) on agar plate culture

Conclusion:

The findings of the present study showed the highest sensitivity with Baermann method, and the lowest with Harada-Mori. Several studies have shown that agar plate method has the most sensitivity (4, 13, 14) while this study showed the opposite. The two methods of Agar Plate and Baermann are recommended for the diagnosis of *S. stercoralis*, but the agar plate method is 11 times more expensive than Baermann's. In addition, the risk of infection increases due to proliferation of parasites and the probability of penetration of filariform larva from agar plate to the skin (15). Moreover, in some cases with auto-infection, or when in those with chronic infection the parasite multiplies in body, but they do not repel larvae, hence the infected people may not be diagnosed (16). Agar Plate method requires more materials and equipment, and a duration of several days for incubation, particularly an experienced skilled laboratory personnel comparing to Baermann (11, 17).

Baermann method showed the highest sensitivity, which is consistent with the study conducted in western Uganda and China (18, 19). It is probably due to the higher amount of fecal specimens, the high water volume in the funnel which can be centrifuged several times to increase the chance of observing the parasite. Since the number of discharged larvae in the feces is low or variable, the high amount of stool in this method is affected. In some studies, although the agar plate method was more sensitive than Baermann, there have also been reports of a positive Baermann method but have not grown by the agar plate culture (4). Some researchers consider the combination of two methods, Baermann and agar plate, as the diagnostic golden standard, for this reason it is better a combination of different methods of parasitology used when it is suspected, especially with the chronic cases of the disease (2, 16).

In most laboratories, conventional parasitological methods, especially direct smear is used because it is both cost and time effective (13). However, in some people who have chronic infection and their parasitic load is low or excretion of larvae is irregular, at least, three fecal specimens every other day, is required (7, 20). As we have seen, by the direct smear method, frequency of parasites with triple sample has

increased significantly compared to the formalin-ethyl acetate concentration method and its sensitivity placed after the Baermann method (Table 1).

The formalin- ethyl acetate concentration method is not very practical because it is difficult to differentiate dead and degenerative larvae (13). In addition, the larvae of parasites may be trapped in the gauze embedded in the glass funnel. As we have seen, the rate of infection by the direct smear was higher than the formalin-ethyl acetate method.

In the present study, the least susceptibility of the laboratory method belonged to the Harada-Mori method which is consistent to the study of Blatt JM & et al. in Brazil (13). This technique is rarely used in diagnostic medical laboratories as a standard method (7).

The sensitivity of PCR method was low compared to the Baermann or the wet smear method, but it was much more than the agar plate culture which is consistent with a study conducted in Brazil (21).

In this study 9 positive cases were detected by PCR method. Perhaps one of the reasons for the low levels of positive cases was the presence of the inhibitors which can interfere with PCR results (22, 23). It can be effective especially when there are a few number of parasites and low amount of DNA in the stool (24). However, 3 of the samples were positive by this method, and negative with the other parasitological methods. Molecular methods require many and expensive equipment as well as skilled personnel, therefore it cannot be done in every medical diagnostic laboratory.

One of the advantages of this study was the use of five different methods to detect this parasite and the limitation of the study struck on the lack of cooperation of some residents in the center.

Since the standard method for the detection of this parasite has not yet been identified, the attention should be focused on a simple, sensitive and efficient method. However, examination of multiple stool samples instead of a single one resulted in an increase of the observed larva, even with only direct smear method. Highest sensitivity was observed when different diagnostic approaches were combined. Among concentration methods, Baermann was an easy, practical and inexpensive method, therefore it is recommended as a selective method for detecting *S. stercoralis,* particularly in chronic infections.

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آیا روش بائرمن، کاربردی‌ترین روش شناسایی استرانژیلوئیدس استرکورالیس می‌باشد؟

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مجله پزشکی هرمزگان سال بیست و دوم شماره اول ۹۷ صفحات ۷۶-۷۰

چکیده

مقدمه: *برآورد شده که ۱۰۰ تا ۳۰۰ میلیون نفر در جهان به استرانژیلوئیدس استرکورالیس آلوده هستند. اگرچه چندین روش برای تشخیص این انگل وجود دارد، هنوز روشی که استاندارد طلایی باشد، تعیین نشده است. نیاز به روشی کاربردی با حساسیت و اختصاصیت بالا شدیداً احساس می‌شود، به خصوص در افرادی که نقص سیستم ایمنی دارند یا کسانی که داوطلب پیوند عضو هستند.*

روش کار: *حساسیت پنج روش انگل شناسی، شامل اسمیر مستقیم مدفوع در محلول نمکی و لوگول، بائرمن، روش فرمالین -اتیل استات، هاراداموری، آگار پلیت و مولکولی توسط نمونه‌های مدفوع جمع‌آوری شده از مرکز نگهداری عقب ماندگان ذهنی بندرعباس مورد بررسی قرار گرفت که روشی ساده، عملی و مقرون به صرفه برای شناسایی استرانژیلوئیدس استرکورالیس تعیین شود.*

نتایج: *پنج روش انگل شناسی و مولکولی با استفاده از ۱۶۳ نمونه مدفوع مورد بررسی قرار گرفت. با توجه به روش‌های مورد استفاده، ۳۰ مورد استرانژیلوئیدس استرکورالیس یافت شد. نمونه‌های مثبت استرانژیلوئیدس استرکورالیس با استفاده از روش‌های مختلف به عنوان استاندارد طلایی در نظر گرفته شد. بالاترین حساسیت با روش بائرمن ۲۷ (۹۰ درصد) و کمترین حساسیت با روش ها راداموری ۱ (۳/۳ درصد) دیده شد. در این مطالعه، ۹ مورد با روش PCR(۳۰ درصد) شناسایی شد.*

نتیجه‌گیری: *بالاترین حساسیت با استفاده از ترکیبی از روشهای مختلف تشخیصی و سپس با استفاده از روش بائرمن بدست آمد. بنابراین، این روش به عنوان یک روش انتخابی برای تشخیص استرانژیلوئیدس استرکورالیس، به دلیل ماهیت آسان، عملی و ارزان توصیه می‌شود.*

کلیدواژه‌ها: *روش‌ها، استرانژیلوئیدس استرکورالیس، تشخیص*

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