

A Simple Modification of the Baermann Method for Diagnosis of Strongyloidiasis

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The diagnosis of Strongyloides stercoralis infections is routinely made by microscopic observation of larvae in stool samples, a low sensitivity method, or by other, most effective methods, such as the Baermann or agar culture plate methods. We propose in this paper a practical modification of Baermann method.

One hundred and six stool samples from alcoholic patients were analyzed using the direct smear test, agar culture plate method, the standard Baermann method, and its proposed modification. For this modification the funnel used in the original version of the method is substituted by a test tube with a rubber stopper, perforated to allow insertion of a pipette tip. The tube with a fecal suspension is inverted over another tube containing 6 ml of saline solution and incubated at 37°C for at least 2 h. The saline solution from the second tube is centrifuged and the pellet is observed microscopically.

Larva of S. stercoralis were detected in six samples (5.7%) by the two versions of the Baermann method. Five samples were positive using the agar culture plate method, and only in two samples the larva were observed using direct microscopic observation of fecal smears. Cysts of Endolimax nana and Entamoeba histolytica/dyspar were also detected in the modification of Baermann method.

Data obtained by the modified Baermann method suggest that this methodology may help concentrate larvae of S. stercoralis as efficiently as the original method.

Key words: *Strongyloides stercoralis* - Baermann method - agar culture plate method - direct smear - intestinal parasites

Strongyloides stercoralis was used to be restricted to tropical areas in the past, but it is found nowadays in other latitudes, including the south-eastern United States (Liu & Weller 1993). Its clinical relevance increases when one considers its opportunistic behavior in compromised patients, in whom autoinfection is frequent and sometimes leads to chronic infections, which may be fatal due to secondary bacterial infections (Link & Orestein 1999). The diagnosis of strongyloidiasis is still made by the detection of larvae in stool samples by microscopic observation of stool smears. This is a relatively insensitive method, made worse by the fact that this parasite exhibits intermittent shedding of larvae (Dreyer et al. 1996, Uparanukraw et al. 1999). Thus, the prevalence of *S. stercoralis* is often underestimated (Genta 1989). For these rea-

sons, examination of up to seven stool samples from each patient in consecutive days is recommended, using more sensitive methods than direct microscopy observation of fecal smears (Sato et al. 1995).

Several more sensitive methods have been described for the isolation of nematode larvae from stools, such as the Baermann method (BM) and agar plate culture method (APC) (Koga et al. 1990, 1991, 1992). The former is a cumbersome method, whereas the later is easier to perform than the BM, but is more expensive (Kamisky 1993) and requires skillful manipulation of the samples because it carries the risk of percutaneous infection of laboratory personnel (Koga et al. 1990, Kamisky 1993). Both methods are labor-intensive, time consuming, and show similar sensitivity (Kamisky 1993). The BM method is more frequently used, but the use of a funnel or a flask to deposit the fecal material from each sample is cumbersome. This may explain why this method is not used in most laboratories, and why the prevalence of strongyloidiasis is often underestimated. An easy and practical modification of the BM proposed in this paper may contribute to facilitate and optimize the diagnosis of strongyloidiasis.

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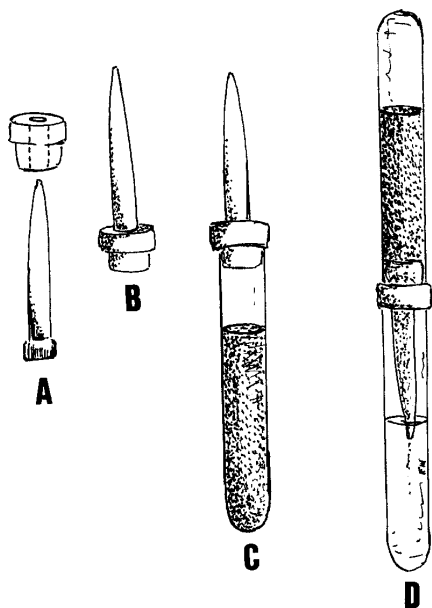
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MATERIALS AND METHODS

In our proposed modification of the BM, the funnel with the hose and Mohr clamp is replaced by a 16X100 mm test tube with a rubber stopper that is perforated to allow introduction of a plastic (Rainin P1000) pipette tip (Fig. A). A portion of feces (2 g) is deposited in the tube containing 8 ml of saline solution and shaken to make a suspension. Then, the tube is sealed with the tipped rubber stopper (Fig. B), and this tube is inverted on top of another tube containing 6 ml of saline solution at 37°C (Fig. C) and incubated in a 37°C water bath for at least 2 h. The second tube is then centrifuged and the sediment analyzed using light microscopy.

Stool samples from 106 chronic alcoholic patients admitted into an Alcoholic Clinic were collected for routine coproparasitological testing, and analyzed within 3 h after collection, using the modified Baermann method described herein. Results were compared with the standard BM, ACP, and direct smear analysis.



A: a perforated rubber stopper and a plastic (Rainin P1000) pipette tip; B: the pipette tip is pushed through the perforated stopper; C: a fecal suspension is deposited in a test tube and sealed with the tipped stopper; D: the tube with the fecal suspension is inverted on top of another tube with 6 ml of saline solution at 37°C and incubate 2 h.

RESULTS

S. stercoralis larvae were found in six patients (5.7%), as detected by the original and modified versions of the BM. Five were detected in the ACP, and only in two samples the larvae were observed in stool smears (Table). Other parasites found in

the fecal smears of these patients included *Endolimax nana* (16%), *Giardia duodenalis* (7.5%), *Entamoeba coli* (6.6%), and *E. histolytica/dyspar* (4.7%). Also, one case of each of the following parasites was found: hookworm, *Hymenolepis nana* and *E. hartmannii*, representing a prevalence of 0.9%, respectively.

Thirty two out of 106 patients (30%) were found to have at least one kind of intestinal nematode or protozoan. Some of these intestinal parasites were detected only by our proposed modification of the BM, as in five out of the twelve cases of *E. nana* diagnosed, and the three cases of *E. histolytica*. Cysts of *E. hartmannii* and *G. duodenalis* from one patient were detected only by fecal smears.

TABLE

Detection of *Strongyloides stercoralis* by the Baermann method, its modification, agar culture plate, and direct smear in 106 alcoholic patients

Diagnostic method	Positive tests/ <i>S. stercoralis</i> positive patients	Negative tests/ <i>S. stercoralis</i> negative patients
Baermann	6/6	100/100
Baermann modified	6/6	100/100
Agar culture plate	5/6	101/100
Direct smear	2/6	104/100

DISCUSSION

The BM and ACP are two of the recommended techniques for the diagnosis of *Strongyloides* infections. The BM is almost 11 times cheaper than the ACP. Also, an inexpensive modification of the BM was published by Graeff-Teixeira et al. (1997), in which they made a funnel with a plastic bottle. However, they are cumbersome methodologies. Whereas the ACP also poses some added difficulties beyond its cost, such as the skillful manipulation of the cultured plates to wash the larvae and the risk of percutaneous infection (Koga et al. 1990, Kaminsky 1993). However, both methods have good sensitivity. In our samples we found that the BM was the most sensitive, and there were no differences between the standard method and our proposed modification. Also, the specificity of the two versions of the BM, the agar culture plate, and direct smear, is higher than 90%, because the correct identification of larvae is an easy task in microscope preparations obtained by these methods. Troublesome features of BM, such as the space required in the laboratory to perform the routine analysis of fecal samples are obviated by the proposed modification, because all that is needed to process each sample is a test-tube. It is possible to

incubate several samples in a standard test-tube rack. Also, the amount of feces deposited in each tube is similar to that used in the ACP, and easier to manipulate.

In the modification of the BM described here the larva of *S. stercoralis* migrate into the second test-tube due to its thermotropism and pushed by gravity. This gravity sedimentation explains the finding of other parasites, such as cysts of protozoa, that were not detected in direct smears but were observed in the slide preparation from our modification of BM.

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