SHORT REPORT: A MODIFIED FILTER PAPER CULTURE TECHNIQUE FOR SCREENING OF STRONGYLOIDES STERCORALIS IVERMECTIN SENSITIVITY IN CLINICAL SPECIMENS

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Abstract. An in vitro test for the detection of ivermectin sensitivity of Strongyloides stercoralis in human clinical specimens was developed. The test has a simple procedure that combines parasite culture with a drug assay. It is based on the principle of the drug effect on larval development test, combined with a modified coproculture using the filter paper culture technique. This does not require parasite isolation, and drug sensitivity can be shown in relationship to the dose. The test can be applied in field surveys for anthelmintic sensitivity and for appropriate drug rotation strategies when drug resistance occurs.

Strongyloides stercoralis is a nematode that infects several million people worldwide.1 Strongyloidiasis is a serious threat to public health in tropical and subtropical areas.2 The clinical spectrum of strongyloidiasis varies from asymptomatic infection, to mild symptomatic abdominal and skin diseases, to fatal disseminated infection in immunosuppressed patients.2–4 Albendazole and ivermectin are the anthelmintic drugs generally used to treat human strongyloidiasis.2,5 Presently, the drug of choice against S. stercoralis is ivermectin, although many reports revealed an incomplete cure.2,6–9 The cure rates ranged from 67% to 100%. Evidence of ivermectin resistance has also been shown for Haemonchus contortus and Trichosstrongylus colubriformis in South Africa, South America, the United States, and Australia.10 Therefore, a validated test for ivermectin sensitivity needs to be used for assessing an emerging resistance of S. stercoralis.

Several tests are in use for the determination of drug resistance of veterinary helminths.11 An in vitro assay for the detection and monitoring of anthelmintic sensitivity in Strongyloides species has also been developed.12 This assay is based on the assessment of drug effects on the larval motility of the infective stage.13 The aim of this study was to develop a simple in vitro assay for determining drug sensitivity of human S. stercoralis from clinical specimens in field conditions. The study was approved by the Human Ethics Committee of Khon Kaen University. Informed consent was obtained from all adult participants and from parents or legal guardians of minors. Stool samples were collected from 95 S. stercoralis–infected subjects in Kalasin and Khon Kaen Provinces, north-east Thailand. These areas are known to be endemic for S. stercoralis, with a prevalence of 23.5%.13 The parasitological diagnosis was based on the detection of S. stercoralis larvae by agar plate culture technique,14 direct saline smear method,15 and quantitative formalin ethyl acetate concentration technique.16 Fecal samples that were positive for S. stercoralis were processed further. Samples with mixed hookworm infection were excluded from the study.

This assay combined the principle of the drug effect on larval development test with a modified coproculture using the filter paper culture technique.15 The results were based on the motility of the infective-stage larvae from coproculture. Briefly, 1 g of feces was smeared in the center of a narrow filter paper strip and placed in a glass test tube (15 mm × 150 mm). Five milliliters of either various concentrations of an ivermectin (for animal use; The British Dispensary, Samut-prakarn, Thailand) solution (30, 15, and 7.5 μg/mL) or of the control (distilled water) was added to the bottom of each tube at 25°C. All tests were performed using duplicate tubes. On the third and fifth days of culture, the tubes were examined for the effect of the drug on worm viability based on the motility of the infective stage larvae at ×40 magnification. All control (no drug) tubes showed motile worms, and the single infection with S. stercoralis was reconfirmed under ×100 microscopic magnification. The criterion for drug resistance was the demonstration of at least one motile worm in duplicate tubes under ×40 magnification either after 3 or 5 days of culture. Dose–response data were analyzed using linear regression (SPSS for Windows version 11.0; SPSS, Chicago, IL).

The results revealed that this in vitro larval development assay was capable of assessing ivermectin sensitivity of S. stercoralis larvae in clinical specimens as well as the dose response (Table 1). Combining the dose–response data after 3 or 5 days of culture with increasing drug concentration, the percentage of worm survival decreased as follows: 46/95 (48.4%) at 7.5 μg/mL, 30/95 (31.6%) at 15 μg/mL, and 13/95 (13.7%) at 30 μg/mL, respectively (R = −0.985), showing that the ivermectin sensitivity of the tested S. stercoralis populations was indeed related to dose. It could be argued, however, that the genetic changes leading to reduced sensitivity do not produce worms that are less fit with the dose range. This problem can be clarified by extending the range of drug concentrations to fit with individual subjects.

To maintain the specificity and to ensure that the positives at higher concentrations are not caused by hookworms or free living nematodes, it is recommended to confirm the diagnosis by aspirating the worm content and examining it under the microscope.

The assay described here has several useful features for testing drug sensitivity. It requires a small fecal sample, and it has a simple procedure combining parasite culture and drug assay systems. This reduces the time of the assay and does not require parasite isolation. The test is simple and therefore

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possibly applicable in field surveys for anthelmintic sensitivity. Moreover, the information obtained about drug sensitivity can be used for appropriate drug rotation strategies. In addition, this method may be suitable for identifying resistant strains that can be further studied for molecular markers of resistance. This method possibly reflects the \textit{in vivo} situation; however, the correlation between the results of the \textit{in vitro} assay and the drug response \textit{in vivo} needs further study.

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