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Evaluation of a New Method for Routine In Vitro Cultivation of *Giardia lamblia* from Human Duodenal Fluid

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Although a method for in vitro cultivation of *Giardia lamblia* was described as early as 1927, only a few clinical isolates were cultured in vitro due to the complexity of the techniques. We developed a method which allows for the routine isolation of *Giardia* trophozoites from human duodenal fluid and maintenance of the organisms in axenic culture. This study evaluates the method in 198 patients. Seventeen strains of *Giardia* were isolated and cultivated axenically. The method was more sensitive than the microscopic examination of aspirated fluid and examination of an impression of mucosal biopsy. Five patients, however, excreted cysts in the stool, although no trophozoites could be demonstrated in the duodenal fluid. *G. lamblia* were cultivated from one patient who did not excrete cysts. The method will enable the collection of *G. lamblia* strains from clinical material in large numbers and can offer an important advance in epidemiological, biochemical, immunological, and therapeutic investigations of giardiasis.

Giardia lamblia was the first protozoon associated with human disease. For more than 300 years, its presence has been described all over the world. Since van Leeuwenhoek's finding, scientists have often cast doubt on the pathogenicity of *G. lamblia* because of the sometimes vague and intermittent aspect of the disease. However, the parasite affects immunosuppressed patients (1) as well as healthy children, adults, and travelers (6). It causes massive epidemics (29) in closed groups such as health- and day-care centers (5) or in the community and can even infect entire villages (27). Only fundamental research on the pathogenic mechanism of *G. lamblia* can elucidate the reasons for the wide variety of symptoms attributed to this microorganism.

Meyer and Radulescu (21) suggested in 1984 that the in vitro cultivation of the microorganism is of particular interest since the study of a large number of strains, especially those of human origin, could yield insight into their classification and epidemiology. A method consistently permitting the routine isolation of the organism from the host, unavailable until now, would therefore be most helpful.

Successful attempts to grow the parasite were reported as early as 1927 (7), 1929 (23), and 1931 (24). Karapetyan grew living trophozoites symbiotically with chicken fibroblasts and *Candida guilliermondii* in 1960 (15) or with *Saccharomyces cerevisiae* from rabbits (16). Meyer succeeded in growing trophozoites axenically in rabbits, chinchillas, and cats (19) in 1970 and later on in human duodenal fluid (20). These methods however presented major disadvantages. The complexity of the techniques and the preparation of the media made it unsuitable for application outside of a research laboratory.

Excystation of *Giardia* cysts recovered from stools (4) was used to recover several strains of human and nonhuman *Giardia* isolates and to grow them axenically (3). This procedure however was very time consuming, and the success rate was low. Moreover, as the excystation often fails, the selection of certain strains that survive the excystation cannot be excluded.

Because for these reasons recovery of clinical isolates on a large scale remained impossible, the aim of our work was to develop a technique for routine in vitro cultivation of *G. lamblia* trophozoites from human samples.

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MATERIALS AND METHODS

Culture medium. The medium we developed is based on the TPS-1 medium described by Diamond (8). The medium was originally derived from the TP medium for the culture of amoeba (13). Visvesvara (30) further modified the Diamond formula with horse serum. Although some ingredients are hard to find, this medium seems richer than the more recently described TYI-S-33 medium (17). It contains (among other things) trypticase peptone (11921; BBL Microbiology Systems, Cockeysville, Md.), panmede liver digest (L 27; Oxoid Ltd., London, England), glucose (8342; Merck), L-cysteine (2838; Merck), and ascorbic acid (127; Merck). We supplemented it with 10% fetal calf serum instead of horse serum and sterilized it through a Seitz EKS no. 6 filter. After sterilization, we added 10% NCTC 135 solution with L-glutamine (041-1350H; GIBCO Ltd., Paisley, Scotland) under laminar flow. Subsequently, the following antibiotics (concentration) were added: penicillin (100 IU/ml), streptomycin (100 mg/liter), vancomycin (20 mg/liter), and clindamycin (20 mg/liter). The final solution was dispensed under laminar flow in sterile 10-ml bottles (VirTis 10-156-10) and lyophilized aseptically (11). The bottles were sealed with rubber plugs and secured with aluminum seals. The medium, prepared and lyophilized as described above, is now produced commercially by the Institut Virion, CH-8803 Rüschlikon, Switzerland.

Duodenal fluid. A sample of duodenal fluid was obtained from all 198 patients who underwent duodenal intubation. All patients were children, 6 months to 6 years old, and were referred to the Department of Pediatric Gastroenterology because of growth retardation or chronic gastrointestinal

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TABLE 1. Comparative results of the *G. lamblia* culture method and other diagnostic tests in 198 consecutive duodenal fluid aspirations

G. lamblia culture (no. of patients)	Presence of *G. lamblia* detected (no. of cultures) with other diagnostic tests						
	Microscopy of duodenal fluid		Microscopy of mucosal print		Stool microscopy		
	+	−	+	−	+	−	ND[a]
Positive culture (17)	16	1	16	1	15	1	1
Negative culture (176)	0	176	0	176	5	73	98
Contaminated culture (5)	0	5	0	5	0	0	5

[a] ND, Not done.

symptoms or both. A pediatric Watson capsule modified with a double lumen tube was inserted into the duodenum and monitored by radioscopy. Once the capsule was properly positioned, duodenal fluid was aspirated freely, and a biopsy of the small intestinal mucosa was taken. After 1 to 2 ml of fluid was obtained, the capsule was gently withdrawn. Immediately after aspiration, about 0.1 ml of the fluid was inoculated into the medium, as described below.

The remaining quantity of the fluid was examined simultaneously for the presence of *G. lamblia* trophozoites under the microscope.

A smear of the impression of the mucosal biopsy on a glass slide was fixed, Kohn stained, and examined microscopically for the presence of trophozoites in all patients.

One or more stool samples was obtained from 94 patients and screened for the presence of *G. lamblia* cysts by a concentration flotation technique and microscopic examination (2).

Inoculation of the medium. Prior to use, the bottles containing the lyophilized medium were rehydrated by injecting about 10 ml of sterile distilled water at room temperature through the rubber plug and filling the bottle to the top. About 0.1 ml of duodenal juice was injected through the stopper into the culture bottle. The syringe was flushed by aspirating some broth and reinjecting it into the bottle. Subsequently, the medium was incubated at 37°C as soon as possible. The bottles were screened for living *G. lamblia* trophozoites after 2 h of incubation with a Leitz inverted microscope at a magnification of ×63. When the concentration of trophozoites in the duodenal fluid was high, living *G. lamblia* organisms could already be detected in the medium on arrival in the laboratory. Cultures were incubated for 10 days and inspected every day.

Storage of *G. lamblia* strains. When *G. lamblia* trophozoites were detected, the medium was further incubated until a concentration of about 10^6 organisms per ml was reached (as counted in a Fuchs-Rosenthal chamber) and subsequently reinoculated.

For this purpose, 3 to 4 ml of broth was aspirated from the original culture bottle and reinoculated in a 16-ml screw-cap glass tube, containing the same medium but without clindamycin or vancomycin. In this medium, the axenic culture was maintained until storage. The strains were cryopreserved (18) and stored in liquid nitrogen.

RESULTS

Table 1 shows the comparative results of the different methods. Altogether, 21 of the 198 (10.6%) patients examined showed evidence of *G. lamblia* by one or more of the methods used.

In 17 cases, trophozoites grew in the culture medium. Trophozoites were seen on direct microscopic examination of 16 duodenal fluid samples (8.1%), and examination of the stained mucosal impression correlated fully with this finding (16 of 16 strains).

The new cultivation method yielded growth of 10^5 living organisms per ml in less than 72 h for all 16 strains. In the one remaining case, the culture became positive, although microscopic examination of both the Kohn-stained mucosal impression and of the duodenal juice were negative. However, growth was only detected in this bottle after 10 days of incubation.

Microscopy of fecal material was performed in 94 cases (47.5%). Twenty patients were found to have excreted *G. lamblia* cysts. In five of them, no trophozoites could be demonstrated in the duodenal aspirate. In the stool of one patient harboring *G. lamblia* in the duodenum, no cysts could be demonstrated.

DISCUSSION

The diagnosis of giardiasis is often difficult. A high rate of false-negative results occurs in the commonly used microscopic examination of stools for the presence of cysts as well as in other diagnostic tests (14). In this study, a higher rate of positivity was found by stool examination than by the other methods used. This finding coincides with the results of some previous studies (22). This might be explained by the patchy nature of more distal loci of infection in the small intestine (31).

Although improving the diagnosis of giardiasis was not the main aim of the work, the comparative results demonstrate the culture method to be at least as sensitive as microscopic examination of duodenal fluid or mucosal print. Moreover, one patient in our study was found to harbor the parasite in the duodenum but persistently failed to excrete cysts. Although some investigators report the examination of duodenal fluid to be more reliable than stool examination (14), we feel that aspiration of duodenal fluid as well as subsequent attempts to cultivate the parasite should supplement but not replace the stool examination. Nevertheless, the ability to grow the infecting strain in vitro permits sensitivity testing (9, 10, 12) and the study of defense mechanisms of the individual patient to the strain (28). Therefore, the culture method may contribute to the management of chronic or recurrent giardiasis.

Although a good medium was described more than 20 years ago, an easy method for the routine cultivation of *G. lamblia* of human origin was not available. The technique presented in this study therefore may prove to be an important advance in fundamental *Giardia* research.

The achievement of the in vitro culture since 1960 (15) has enabled the development of antisera (S. O. Osipova, N. A. Dekkan-Hodjaeva, L. V. Medvedena, and A. P. Babakaev. Proceedings of the Third International Congress on Parasitology 2:1099–1100, 1974) and the detection of specific antibodies in the host (25). Biochemical and growth charac-

teristics of the organism have been studied, and animal models have been developed (26). Nevertheless, to isolate *G. lamblia* routinely and to grow and collect isolates from different sources on a large scale is essential for future research projects. The in vitro susceptibility of the isolates to experimental antiparasitic drugs can be determined. Differences between species can be investigated and may contribute to their classification. Antigenic structure analysis and immunological investigations of the isolates eventually may lead to the description of different serological types, facilitating epidemiological studies. Examination of the immunological properties of a large number of *Giardia* strains is also important to test the specificity of antisera and to evaluate a method for antigen detection.

Because the method described in this study is simple to perform and will be available commercially, we feel it may serve as a base for future *Giardia* research projects.

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