**PHILIP D. BUTCHER and MICHAEL J. G. FARTHING**

**Department of Gastroenterology, St Bartholomew’s Hospital, West Smithfield, London EC1A 7BE, U.K.**

*Giardia lamblia* is the most frequently identified intestinal protozoan parasite in the U.S.A. and U.K., and is one of the more common causes of diarrhoea in children in the developing world. Chronic infections can result in enteropathy and malabsorption, contributing to growth retardation in children (Farthing et al., 1986). *Giardia* is commonly found in patients with acquired immunodeficiency syndrome and in other immunocompromised individuals. An important practical problem in the management of this infection is that diagnosis is not straightforward. Diagnosis of giardiasis relies at present mainly on the demonstration of *Giardia* cysts in stools by microscopy. Cyst excretion is known to be erratic, such that a single faecal specimen may only detect 50% of cases (Burke, 1975), while sequential stool analysis only decreases the false negative rate to 30% (Burke, 1977). Duodenal aspiration and biopsy followed by microscopy for *Giardia* trophozoites are only reportive in 44% of cases (Madanagopalan et al., 1975). Serodiagnosis by measurement of serum anti-*Giardia* IgM levels appears to be useful in acute giardiasis but IgG levels do not discriminate current from previous infections (Goka et al., 1986).

Clearly there is a need for a simple, rapid, sensitive and specific diagnostic technique that can detect *Giardia* in faecal specimens. The techniques of gene cloning as applied to infectious diseases now allow the production of gene probes that have the potential to be exquisitely sensitive and specific diagnostic reagents. Increasing numbers of gene probes for bacterial and parasite infections are rapidly being developed and exploited (Tenover, 1988). We report here the generation of cloned genomic DNA probes for *Giardia lambia* and their application to the diagnosis of human giardiasis by detection of *Giardia* DNA in faeces.

A fully representative BamHI-digested genomic DNA library of *G. lamblia*, strain Portland I, was constructed in *Escherichia coli* XL-1 Blue host cells with the plasmid vector pBluescript (Stratagene L-TD, NBL U.K.) consisting of >10⁶ clones. Cloning efficiencies of 10³ clones/μg of *Giardia* DNA were achieved. Recombinant clones were selected on ampicillin plates by blue/white colour selection in the presence of X-Gal after isopropyl β-D-thiogalactopyranoside induction of the plasmid lacZ gene β-galactosidase fusion protein. Initial screening of 2000 white clones was performed by colony hybridization in situ with [32P]dCTP-labelled probes specific for *Giardia* DNA insert. No hybridization was observed with DNA from other protozoal or human DNA. This confirmed the origin and specificity of the selected *Giardia* clones. A single clone, pGII-2, with a 2 kilobase insert was selected and the insert excised with BamHI, separated by electrophoresis in low melting temperature agarose and labelled with [35P]dCTP (Amersham; 3000 Ci/mmol) by random hexanucleotide priming and Klenow extension (Fienberg & Vogelstein, 1983). Specific activities of 4·8 x 10⁶ c.p.m./μg of DNA were achieved. This was used as the probe to detect *Giardia* DNA in faeces by dot-blot hybridization.

Faecal samples from 21 confirmed cases of giardiasis, containing between 10³ and 10⁷ cysts/g of stool (Hospital for Tropical Diseases, London), and four negative control samples were studied. Faecal suspensions (1 g/2 ml of water) were made and centrifuged at 10000 g for 10 min. The supernatant containing soluble DNA was removed. The pellet was washed free of bacteria in 10 vol. of water and centrifuged at 1500 g for 10 min. This fraction was the crude washed faecal material containing cysts. Enriched cyst fractions were prepared by sucrose flotation (Bingham et al., 1979). DNA extraction from crude pellets and enriched cyst preparations was by lysis in 1% (w/v) SDS in TEN buffer (50 mm-Tris/HCl, pH 8.0, 100 mm-EDTA, 150 mm-NaCl) and digestion with 500 μg of Proteinase K/ml at 55°C for 3 h. Nucleic acid was recovered by phenol/chloroform extraction and ethanol precipitation and redissolved in TE (10 mm-Tris-HCl, pH 8.0, 1 mm-EDTA). Soluble fractions and DNA extracts from 0.1 g of faeces (200 μl) were made 10 × SSC (1 × SSC = 0.15 M-NaCl, 0.1 M-sodium citrate, pH 7.0), de-natured at 100°C for 5 min, chilled and 500 μl of unlabeled nylon filters (Hybond; Amersham) using a dot-blot apparatus (Biorad). DNA was cross-linked by u.v. irradiation and baked for 1 h at 80°C. Hybridization of dot-botted faecal fractions with radiolabelled pGII-2 insert DNA was at 65°C for 18 h in 6 × SSC, 5 × Denhardt's, 1% (w/v) SDS and 100 μg of denatured salmon sperm DNA/ml, after preincubation for 4 h without probe (Maniatis et al., 1982). Filters were washed in 1 × SSC/0.1% (w/v) SDS at 65°C for 3 × 15 min and autoradiographed.

Sensitivity calibration of the detection of *Giardia* DNA was performed by dot-blotting dilutions of total *Giardia* DNA containing 0.1 ng to 1 μg of DNA. A minimum of 1 ng of DNA was detectable, equivalent to about 5 x 10³ *Giardia* trophozoites based on a genome size of 3·2 x 10⁸ base pairs (Nash et al., 1985). No *Giardia* DNA was detected in the soluble faecal fraction or crude washed pellets of any of the samples tested. However, DNA from a minimum of 1 x 10³ enriched cysts was readily detectable. Comparisons based on the strength of hybridization signal of pure *Giardia*...
DNA with those obtained from cyst DNA preparations indicate that the yield of DNA from lysis-resistant cysts was < 5%.

The results demonstrate that Giardia species-specific cloned DNA probes can detect Giardia DNA in cyst preparations but not soluble DNA in faecal suspensions. Present levels of detection of cyst DNA by hybridization is of little diagnostic potential as an alternative to cyst identification by microscopy. Increased sensitivities are required and must rely on higher yields of cyst DNA by novel approaches to cyst lysis. Diagnosis by detection of soluble DNA in faeces is potentially of most benefit, but present methods are not sensitive enough. Gene amplification by the polymerase chain reaction (Scharf et al., 1986) of specific target sequences may be the way forward.

This work was supported by The Wellcome Trust.


Identification of bacteria using rRNA-gene-specific cDNA probes

NIGEL COX,* JULIE JOHNSTON,* DAVID J. M. WRIGHT† and LEONARD C. ARCHARD*
Departments of *Biochemistry and †Medical Microbiology, Charing Cross and Westminster Medical School, Fulham Palace Road, London W6 8RF, U.K.

Traditionally, bacteria have been identified by their phenotype, but these may not be stably expressed and methods vary between laboratories. Recently, analytical techniques in chemistry and molecular biology have been applied to bacterial identification. Of the various chemical components studied, only chromosomal DNA and RNA composition are unaffected by growth conditions. However, problems are also encountered in DNA studies. In plasmid analysis, plasmids may be unstable; restriction digests of total DNA generate too many fragments for easy comparison and genomic probes are too specific for screening many organisms.

Attention has recently focused on ribosomal RNA genes. These are ubiquitous and highly conserved. rRNA transcript-

![Fig. 1. Autoradiograph of Southern blots probed with rRNA-gene-specific cDNA probe](image)

(a) HindIII-digested DNA. Lane 1, clinical isolate; lanes 2–9 NCTC strains (1, H. parahaemolyticus; 2, A. equuli; 3, P. ureae; 4, P. pneumotropica; 5, P. multiocida; 6, P. gallinarum; 7, P. haemolytica T10; 8, P. haemolytica type strain; 9, P. haemolytica A1). (b) HindIII- and (c) ClaI-digested DNA of three clinical isolates of C. jejuni biotype 1. The isolates are distinguished by their HindIII restriction pattern.

Received 21 September 1988