it becomes increasingly significant when the levels are elevated. It is important to determine the proportion of cholesterol derived from LDL and Lp(a) independently because it is well established that many cholesterol-lowering agents (e.g., statins) are effective in lowering plasma LDL-C but have little or no effect on Lp(a). □

References

1 Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *JAMA* 2001; **285**: 2486–97.

2 Seman LJ, Jenner JL, McNamara JR, Schaefer EJ. Quantification of lipoprotein (a) in plasma by assaying cholesterol in lectin-bound plasma fraction. *Clin Chem* 1994; **40**: 400–3.

3 Scanu AM. Lipoprotein (a), Friedewald formula and NCEP guidelines. *Am J Cardiol* 2001; **87**: 608–9.

Molecular typing of *Dientamoeba fragilis*

J. J. WINDSOR*, C. G. CLARK† and L. MACFARLANE*.

**National Public Health Service for Wales Aberystwyth, Bronglais Hospital, Aberystwyth, Ceredigion; and †Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK*

Dientamoeba fragilis is a human intestinal protozoan parasite that was described originally by Jepps and Dobell in 1918.[1] Initially it was thought to be an amoeba but subsequent studies showed that it shared antigens with the flagellates Histomonas and Trichomonas.[2,3] Transmission electron microscopy (TEM) studies confirmed many trichomonad-like aspects of *D. fragilis* ultrastructure and accordingly it was reclassified to reflect this.[4] However, it was not until Silberman *et al*.[5] sequenced the small subunit ribosomal RNA (rRNA) gene of *D. fragilis* that it was shown conclusively to be a trichomonad, albeit one without a flagellum.

D. fragilis remains a little-studied parasite despite numerous reports linking it with gastrointestinal symptoms.[6–9] This may be explained in part by the difficulties associated with the diagnosis of *D. fragilis* infection, and certainly many laboratories in the UK do not use suitable methodologies for its detection.[10] *D. fragilis* does not have a known cyst stage, and, as the nuclei are not visible in saline or iodine preparations, the trophozoite is difficult to detect in direct microscopy.[11] Permanently-stained smears or faecal culture techniques are the recommended detection methods for *D. fragilis*.[12]

D. fragilis has a worldwide distribution and is one of the most common intestinal protozoa when suitable methods are employed.[12] The numbers of *D. fragilis* detected and reported to the Communicable Disease Surveillance Centre (CDSC; www.hpa.org.uk/cdr/) in England and Wales remain relatively low, although this is likely to be a gross underestimation of the true prevalence. In a previous report, using a sensitive culture method, *D. fragilis* was detected in 2.6% (25/976) of unselected specimens.[13] One of the advantages of using the culture method is that isolates can be typed subsequently using molecular methods.

Johnson and Clark[14] analysed *D. fragilis* ribosomal genes using a polymerase chain reaction (PCR)–restriction fragment length polymorphism (RFLP) technique (riboprinting). They found evidence for two genetically distinct variants; however, the number of isolates investigated was small (n=12). The degree of divergence (about 2%) between these variants is comparable to that between the pathogenic *Entamoeba histolytica* and the non-pathogenic *E. dispar* (which are morphologically indistinguishable). In view of this it seems plausible that the existence of genetic variants might be associated with the variable symptomatology of infection.

In the present study, *D. fragilis* isolates are typed using riboprinting to investigate further the degree of genetic diversity in a larger sample size, in order to add to the limited data available.

All faecal specimens sent to NPHS Aberystwyth were cultured routinely for intestinal protozoa using Robinson's culture method.[15] Over an 18-month period, 43 positive *D. fragilis* cultures were obtained. All were confirmed as *D. fragilis* using a trichrome stain after fixation with Schaudinn's fixative. Cells were pelleted and lysed in

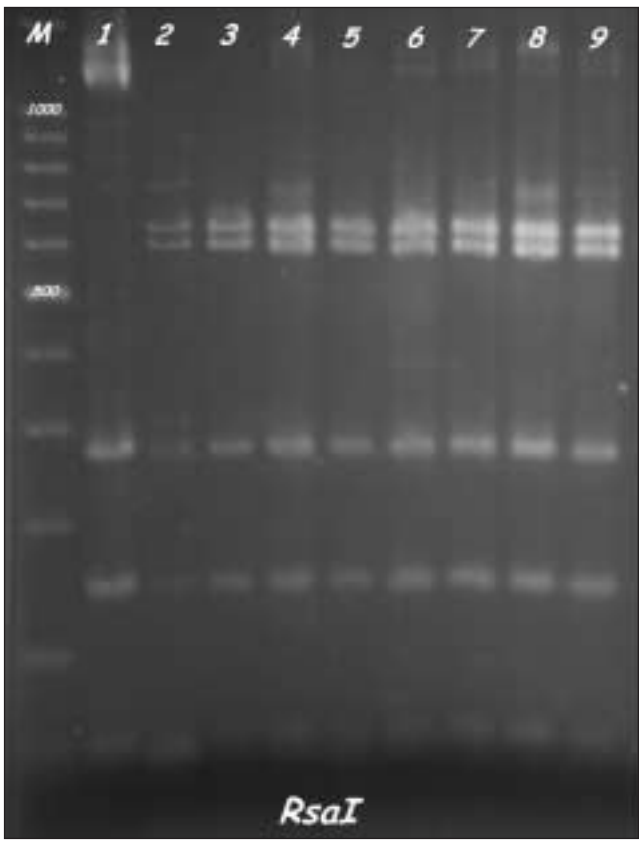

Fig. 1. *Rsa*I digest patterns of *D. fragilis* PCR-amplified SSU-rDNA. M: 100-bp molecular weight marker. Lane 1: genotype 2 (Bi/pa). Lanes 2–9: all genotype 1 (NPHS Aberystwyth isolates).

Correspondence to: Jeff Windsor
NPHS Aberystwyth, Bronglais Hospital, Caradoc Road, Aberystwyth, Ceredigion SY23 3HF
Email: jeff.windsor@nphs.wales.nhs.uk

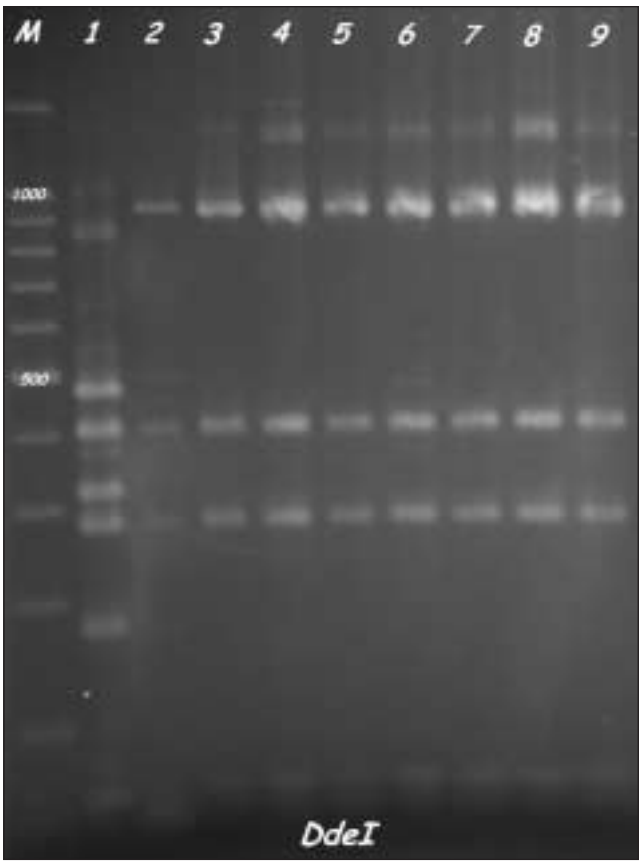

Fig. 2. *Dde*I digest patterns of *D. fragilis* PCR-amplified SSU-rDNA. M: 100-bp molecular weight marker. Lane 1: genotype 2 (Bi/pa). Lanes 2–9: all genotype 1 (NPHS Aberystwyth isolates).

0.25 mL 0.25% sodium dodecyl sulphate in 0.1 mol/L EDTA (pH 8). DNA was extracted as previously described.[16]

Primers TRD5 (GATACTTGGTTGATCCTGCCAAGG) and TRD3 (GATCCAACGGCAGGCCAAGG)[5] were used under standard conditions for PCR amplification. These only amplify trichomonad small-subunit rRNA genes (SSU-rDNA). Amplification was achieved using 30 cycles of 94°C for 1 min, 55°C for 1.5 min, and 72°C for 2 min. The PCR amplification was validated with positive and negative controls. A 1.7-kbp PCR product was produced, digested with the restriction enzymes *Rsa*I and *Dde*I, and the fragments separated in 1.5% agarose gel.

The SSU-rDNAs were amplified successfully from 33 of the 43 lysates studied. Five lysates did not produce an amplification product, and in a further five the amount of product was too small to permit typing. This is likely to reflect inherent problems associated with DNA extraction from samples derived from faeces. Whenever possible, positive cultures were subcultured in an effort to limit the effect of any potential inhibitors that might be present.

However, some positive cultures only contained small numbers of *D. fragilis* and these were not subcultured successfully. The 33 amplified *D. fragilis* DNAs all produced the SSU-rDNA 1.7-kbp amplification product. After digestion with the restriction enzymes, all 33 were found to give the same pattern (genotype 1; Figs. 1 and 2). This is the more common of the two types described by Johnson and Clark.[15] The rarer genotype (genotype 2) has only been found in two cases: a two-year-old child from the UK with no history of travel, and the Bi/pa isolate studied by Camp *et al*.[4] and Silberman *et al*.,[5] which was isolated in Illinois in 1948.

Recently, Peek *et al*.[17] amplified *D. fragilis* directly from human stool specimens using a single-round PCR method. They designed specific Dientamoeba primers, DF1 and DF4, which amplified the region from positions 100 to 761 of the SSU-rDNA. The amplicon produced was much smaller than the complete coding region but the two known genetic variants could still be distinguished using the restriction enzyme *Dde*I. Microscopy-positive *D. fragilis* specimens were analysed from symptomatic patients (n=53; 20 children, 24 adults, and nine travellers) and asymptomatic carriers (n=6). All 59 PCR-positive samples were assigned to the more common genotype 1.

The results of the present study confirmed the findings of Peek *et al*.[17] and showed that *D. fragilis* displays very little variation in its SSU-rDNA, and that genotype 2 is rare. The Bi/pa isolate (genotype 2) is the culture deposited with the American Type Culture Collection (ATCC) and has been the source of material for all *D. fragilis* studies undertaken to date. However, in view of the degree of sequence divergence between the ribosomal genes of the two genotypes, results obtained with Bi/pa may not be representative of the entire species.

Although riboprinting has been used to great effect in distinguishing *E. histolytica* from *E. dispar*[18] and in typing *Blastocystis hominis*,[19] among other applications, it would appear to have limited value with *D. fragilis*. However, more data are needed from patients with different symptomatology (e.g., irritable bowel disease, acute and chronic diarrhoea) and from different geographical areas to substantiate or refute this observation. □

The authors wish to thank the biomedical scientists at NPHS Aberystwyth for collecting and pelleting the D. fragilis *isolates used in this study. JJW was supported by an Institute of Biomedical Science grant.*

References

1 Jepps MW, Dobell C. *Dientamoeba fragilis* n.g., n. sp., a new intestinal amoeba from man. *Parasitology* 1918; **10**: 352–67.

2 Dwyer DM. Analysis of the antigenic relationships among *Trichomonas, Histomonas, Dientamoeba* and *Entamoeba*. III. Immunoelectrophoresis technics. *J Protozool* 1974; **21**: 139–45.

3 Dwyer DM. Analysis of the antigenic relationships among *Trichomonas, Histomonas, Dientamoeba* and *Entamoeba*. II. Gel diffusion methods. *J Protozool* 1972; **19**: 326–32.

4 Camp RR, Mattern CFT, Honigberg BM. Study of *Dientamoeba fragilis* Jepps and Dobell. I. Electron microscopic observations of the binucleate stages. II. Taxonomic position and revision of the genus. *J Protozool* 1974; **21**: 69-82.

5 Silberman JD, Clark CG, Sogin ML. *Dientamoeba fragilis* shares a recent common evolutionary history with the trichomonads. *Mol Biochem Parasitol* 1996; **76**: 311–4.

6 Dickinson EC, Cohen MA, Schlenker MK. *Dientamoeba fragilis*: a significant pathogen. *Am J Emerg Med* 2002; **20**: 62–3.

7 Girginkardesler N, Coskun S, Cuneyt Balcioglu I, Ertan P, Ok UZ. *Dientamoeba fragilis*, a neglected cause of diarrhoea, successfully treated with secnidazole. *Clin Microbiol Infect* 2003; **9**: 110–3.

8 Norberg A, Nord CE, Evengard B. *Dientamoeba fragilis* – a

protozoal infection which may cause severe bowel distress. *Clin Microbiol Infect* 2003; **9**: 65–8.
9 Bosman DK, Benninga MA, van de Berg P, Kooijman GC, van Gool T. [*Dientamoeba fragilis*: possibly an important cause of persistent abdominal pain in children]. *Ned Tijdschr Geneeskd* 2004; **148**: 575–9.
10 Windsor JJ, Johnson EH. More laboratories should test for *Dientamoeba fragilis*. *BM J* 1999; **318**: 735.
11 Windsor JJ, Rafay AM. Laboratory detection of *Dientamoeba fragilis*. *Br J Biomed Sci* 1997; **54**: 223–4.
12 Johnson EH, Windsor JJ, Clark CG. Emerging from obscurity: biological, clinical and diagnostic aspects of *Dientamoeba fragilis*. *Clin Microbiol Rev* 2004; **17**: 553–70.
13 Windsor JJ, Macfarlane L, Hughes-Thapa G, Jones SK, Whiteside TM. Detection of *Dientamoeba fragilis* by culture. *Br J Biomed Sci* 2003; **60**: 79–83.
14 Johnson JA, Clark CG. Cryptic genetic diversity in *Dientamoeba fragilis*. *J Clin Microbiol* 2000; **38**: 4653–54.
15 Robinson GL. The laboratory diagnosis of human parasitic amoeba. *Trans R Soc Trop Med Hyg* 1968; **62**: 285–94.
16 Clark CG, Diamond LS. Intraspecific variation and phylogenetic relationships in the genus Entamoeba as revealed by riboprinting. *J Eukaryot Microbiol* 1997; **44**: 142–54.
17 Peek R, Reedeker FR, van Gool T. Direct amplification and genotyping of *Dientamoeba fragilis* from human stool specimens. *J Clin Microbiol* 2004; **42**: 631–5.
18 Clark CG, Diamond LS. The Laredo strain and other *Entamoeba histolytica*-like amoeba are *Entamoeba moshkovskii*. *Mol Biochem Parasitol* 1991; **46**: 11–8.
19 Clark CG. Extensive genetic diversity in *Blastocystis hominis*. *Mol Biochem Parasitol* 1997; **87**: 79–83.

Improved efficiency of a hepatitis C virus antibody testing algorithm in blood donors from Saudi Arabia

A. ABUTALEB, E. ABED, L. QASEM, Z. A. MEMISH* and A. H. HAJEER

*Departments of Pathology and *Infection Prevention and Control, King Fahad National Guard Hospital, PO Box 22490, Riyadh 11426, Saudi Arabia*

Laboratory diagnosis of hepatitis C virus (HCV) infection began a decade ago with the introduction of an enzyme-linked immunosorbent assay (ELISA) technique.[1] Now, highly sensitive third-generation immunoassays that detect antibodies to structural and non-structural proteins in serum are available.

Diagnosis of HCV infection cannot be made on the basis of ELISA alone, as this technique is not sufficiently specific, especially when testing blood donors, and confirmatory testing by recombinant immunoblot assay (RIBA) is required.[2]

Correspondence to: Dr Ali Hajeer,
Division of Immunology, Department of Pathology, P.O. Box 22490
Riyadh 11426, Saudi Arabia
Email: hajeera@ngha.med.sa

Diagnosis is also possible by nucleic acid testing (NAT). Reverse-transcription polymerase chain reaction (RT-PCR) assays are available for the detection of viral RNA in plasma or serum. However, some cases of HCV infection can produce variable NAT results,[3] and this initiated the production of new Centers for Disease Control and Prevention (CDC) guidelines to confirm infection with HCV.[4]

The guidelines suggest an initial ELISA screen followed by confirmatory RIBA and/or NAT testing, and use of the signal-to-cut-off (S/Co) value of third-generation screening methods to help guide the need for supplemental testing. The S/Co results are divided into negative, weak positive and strong positive. The guidelines suggest that samples which test positive by the screening test with a high S/Co ratio do not need a confirmatory test.

This retrospective study, which looked at all Saudi blood donors who tested HCV-positive on screening immunoassay, aims to evaluate the new guidelines in relation to detecting true HCV-positive Saudi donors by confirmatory RIBA testing.

Blood donors at King Fahad National Guard Hospital undergo strict selection based on criteria laid down by the American Association of Blood Banks (AABB) and College of American Pathologists (CAP). Donors complete a questionnaire and are interviewed before donation.

Recently, a third-generation micro-particle enzyme immunoassay (MEIA) for HCV antibody testing was introduced on the Axsym system (Abbott). The assay detects antibodies to structural and non-structural HCV recombinant proteins. This test is US Food and Drug Administration (FDA) approved for the screening of donors for HCV antibodies.

A total of 208 blood donors were recruited for this study. Results were expressed as the S/Co ratio, which is calculated by dividing the sample rate by the cut-off rate. Cut-off rate is calculated from the mean of two index calibrators. The kit uses S/Co <1.0 as negative and ≥ 1.0 as initially positive. All positive samples were repeated in duplicate.

All 208 donors were tested by RIBA (Chiron V3.0 strip blot assay) to confirm the presence of HCV antibodies. Specificity and sensitivity were calculated using the following equations: specificity=true negative x 100/(true negative + false positive); sensitivity=true positive x 100/(true positive + false negative).

Of the 208 blood donors studied, 111 were positive by screening assay (S/Co ≥ 1.0). Supplementary RIBA testing was carried out on all 208 samples; however, none of the 97 donors that were HCV-negative by ELISA gave a positive RIBA result. The results presented in Table 1 suggest that a cut-off value of 1.0 was non-specific and that the majority of positive screening results were false positives.

All 16 samples positive by RIBA gave very high ELISA readings (S/Co >16). In the Saudi population studied, an S/Co value of 1.0 for the MEIA screening assay resulted in specificity of 50.5% and sensitivity of 100%. When an S/Co of 16 was applied to the 111 positive screening assay results, all 16 donors with an S/Co value >16 were positive by RIBA (specificity 100%, sensitivity 100%).

The results presented here suggest a poor correlation between the MEIA screening assay and RIBA results. The Abbott third-generation MEIA assay, run on the Axsym system (Abbott), showed a low specificity for the detection of HCV-infected Saudi blood donors. Furthermore, the