Comparison of the sensitivity of microscopy and culture in the laboratory diagnosis of intestinal protozoal infection

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SUMMARY Concentration of protozoal cysts from faeces by the formol-ether method and faecal culture on Robinson's medium were studied to determine their diagnostic value among patients attending a sexually transmitted diseases clinic in Edinburgh. Of 450 men studied Entamoeba histolytica and Giardia lamblia were identified in the faeces of 10·7 and 5·1% respectively. Thirty (81·1%) of 37 men with E histolytica and 11 (47·8%) of 23 men with G lamblia infections would not have been identified if formol-ether concentration had not been undertaken. Culture of faeces in Robinson's medium led to the detection of a further 11 men with amoebiasis. Oocysts of Cryptosporidium spp were not identified in faecal samples from 130 men.

Over the past decade the sexual transmission of enteric pathogens among homosexual men has been apparent. For example, of 51 homosexual men attending a venereal diseases clinic in New York City 19·6 and 3·9% had amoebiasis and giardiasis respectively; neither Entamoeba histolytica nor Giardia lamblia was found in the faeces of 64 heterosexual men who attended the same clinic. Although doubts regarding the pathogenicity of E histolytica isolated from homosexual men exist, a recent study has shown that homosexually acquired amoebiasis is associated with proctitis.

There is considerable variation between laboratories in the methods used for the detection of intestinal protozoa; some rely solely on the microscopic examination of a saline mount of fresh faeces.

In this study the results of direct microscopy were compared with those of formol-ether concentration of stool samples for the identification of protozoal cysts from men attending a sexually transmitted diseases clinic. In addition, culture was evaluated for its usefulness in the diagnosis of intestinal amoebiasis as a routine procedure in a non-specialist laboratory.

Infection with the coccidian Cryptosporidium spp acquired immune deficiency syndrome (AIDS). This organism can cause self limiting diarrhoea in immunocompetent individuals, but immunodeficient patients, particularly those with AIDS, may develop life threatening infections. The organism can be acquired from infected animals—for example, calves—or from other humans, presumably through the faecal-oral route. As oro-anal contact is practised commonly by homosexual men, it might be expected that the prevalence of Cryptosporidium spp would be greater among these men than among heterosexuals. We therefore examined stool samples for the oocysts of Cryptosporidium spp.

Material and methods

As part of a study of gastrointestinal infection, a single stool sample was obtained from each of 450 consecutive men (345 homosexual, 105 heterosexual) who attended the Department of Genito-Urinary Medicine, Edinburgh Royal Infirmary, between October 1982 and August 1983. Faecal specimens from 130 consecutive patients (85 homosexual, 45 heterosexual men) were examined for cryptosporidium infection.

Specimens from 15 patients with diarrhoea were examined within 30 min of the passage of the stool; faeces from other men were examined within 3 h of passage. All specimens were examined by direct microscopy after formol-ether concentration. Samples were also cultured for amoebae.
DIRECT MICROSCOPY
About 2 mg of faeces was emulsified in 0.05 ml of 0.85% NaCl unstained or stained with iodine. At least 150 microscopic fields were viewed at a magnification of ×600 for cysts in the stained preparations.

Faecal smears from patients with diarrhoea were fixed in Schaudinn's fluid, stained by a modified Gomori's stain,9 and examined at a magnification of ×1200.

CONCENTRATION METHOD
A modification of Ridley's formol-ether method10 was used; faeces were emulsified in 10% formalin and strained through a gauze swab into a centrifuge tube.

CULTURE OF AMOEBAE
Robinson's medium11 was used, but sheep serum was replaced by bovine serum (Oxoid Ltd, Basingstoke, UK). After incubation at 37°C for 48 h, cultures were examined for trophozoites by direct microscopy with and without the addition of iodine solution. When trophozoites were seen a permanent stained smear was prepared as above and identified on the basis of the nuclear characteristics described by Sargeant and Williams.12

Flagellates were identified after staining with iron-haematoxylin.8

METHODS USED FOR THE DEMONSTRATION OF OOCYSTS OF CRYPTOSPORIDIUM spp
Sheather's sugar flotation method for the concentration of oocysts13 was used in the examination of the first 50 stool samples. In addition, thin faecal smears were prepared and stained by a modified Ziehl-Neelsen method.14 In the investigation of the subsequent specimens only stained faecal smears were examined.

Results
Stool samples from 103 (22.9%) of the 450 men contained at least one species of intestinal protozoa.

The Table compares the results of microscopy of a saline mount preparation of faeces with those of microscopy of a concentrated specimen. The superiority of examination of a concentrated specimen is clear. Trophozoites of E histolytica were found in stained faecal smears from two of 15 men with diarrhoea.

The results of culture of the 450 stool samples are also given in the Table. Neither cysts nor trophozoites of Chilomastix mesnili or Retortamonas intestinalis were identified by direct microscopy of the faecal samples. Eleven (22.9%) patients with E histolytica infection would not have been identified if culture had not been undertaken. E histolytica was not grown from the cyst containing stools of two men (4.2%).

Oocysts of Cryptosporidium spp were not identified in the 130 stool samples examined.

Discussion
This small study confirms the value of the formol-ether method of concentration of protozoal cysts from faeces for the detection of intestinal protozoa. If the procedure had not been undertaken E histolytica and G lamblia infections would not have been recognised in about 80% and 50%, respectively, of infected stool samples. Cysts are often present in the faeces in small numbers, particularly if the individual has taken anti-diarrhoeal agents or antibiotics. As cyst excetration is often intermittent and only a single stool sample from each patient was examined, it is probable that the prevalence of protozoal infection in our study group has been underestimated.

In the diagnosis of E histolytica infection the superiority of culture in Robinson's medium over microscopy was clearly shown, and our results confirm the findings of the original workers.11 About a third of the infected men would not have been recognised if culture had not been undertaken. The
Comparison of microscopy and culture in the laboratory diagnosis of intestinal protozoal infection

reason for the failure of the growth of amoebae from two infected individuals is not known, but it may be related to the recent ingestion of antimicrobial agents.

Isoenzyme analysis of isolates of *E histolytica* has shown that only certain types of *E histolytica* are associated with tissue invasion and hepatic abscess formation. Most isolates from homosexual men are of zymodeme type I, which Sargeant et al do not consider to be pathogenic. As *E histolytica* can be associated with proctitis, however, and as hepatic abscess has been reported in a homosexual man (zymodeme not specified), we recommend routine stool examination for *E histolytica* in homosexual men with gastrointestinal symptoms and treatment if *E histolytica* is found.

Culture of intestinal amoebae is easy and can be undertaken in a routine microbiological laboratory. Until experience has been gained in the identification of species, however, it would be useful to confirm specific identities with a reference laboratory.

Although trophozoites of *G lamblia* have been grown in culture from faeces containing cysts, the procedure is complex, not easily reproducible, and not so suitable for routine diagnosis. The diagnosis of giardiasis therefore rests on the demonstration of trophozoites and/or cysts in the faeces, or of trophozoites in jejunal aspirate or mucosal smears.

The prevalence of *Cryptosporidium* spp in the population and its importance as a cause of diarrhoea are as yet unknown. Jokipii et al found oocysts of the organism in 9% of 154 selected stool samples which had been sent to the Department of Bacteriology, University of Helsinki. Casemore and Jackson, working in a public health laboratory in Wales, found *Cryptosporidium* spp in 1-2% of 500 faecal samples from patients with diarrhoea; interestingly, five patients were aged 10 or younger. Although we did not find oocysts of *Cryptosporidium* spp in the faeces of our patients, only 13 of the 130 men had diarrhoea at the time of stool collection. As cryptosporidiosis is a self-limiting condition with transient excretion of oocysts in immunocompetent individuals, some recent infections may not have been identified. Persistent excretion of oocysts and life threatening diarrhoea occurs in immunocompromised patients. As the incidence of AIDS in the USA and in the UK is increasing, it is probable that more cases of cryptosporidiosis will be encountered. There is a need for laboratory workers to be aware of the disease and be familiar with the diagnostic methods available.

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References

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