

Control of an amoebiasis outbreak in the Philippi area near Cape Town

S. Whittaker, T. F. H. G. Jackson, V. Gathiram, L. D. Regensberg

Previous studies in Durban have shown that serological investigations, in combination with iso-enzyme electrophoresis, are invaluable for monitoring the endemicity of pathogenic strains of *Entamoeba histolytica*. We therefore proposed that antibody profiles could be used to detect epidemic situations. An outbreak of amoebiasis in the normally non-endemic Philippi area near Cape Town provided an opportunity for testing this hypothesis. Seven of 9 patients presenting at a district hospital with invasive amoebiasis originated from a single farm in Philippi. Iso-enzyme electrophoresis and serological investigations were used to monitor the endemicity of amoebiasis on 16 of the 49 farms in this district. In an attempt to contain disease transmission all inhabitants on farms from which patients came (including those where cyst-passers were identified) and all seropositive subjects were treated. The antibody profiles proved invaluable for confirming that the farm from which the hospitalised patients originated was the central focus of the outbreak, and also identified subjects infected with pathogenic zymodemes of *E. histolytica* on the adjacent 4 farms. On all 5 of these farms, 62,5 - 100% of seropositive subjects were strongly positive. In contrast weak to negative serological responses occurred on the remaining 11 farms. In addition the success of treatment was indicated by a notable drop in strong seropositive responses on the affected 5 farms to 11,5% within 9 months. The infection pathways implied that the pathogenic strain of *E. histolytica* was introduced into this non-endemic area by a foreigner from an endemic area; this suggests that the pathogenicity of *E. histolytica* is an immutable stable feature.

S Afr Med J 1994; 84: 389-393.

Department of Community Health, University of Stellenbosch

S. Whittaker, B.Sc., M.B. CH.B., F.F.C.H. (C.M.) (S.A.), M.MED. (COMM. HEALTH), M.D.

Amoebiasis Research Programme, South African Medical Research Council (Natal), Durban

T. F. H. G. Jackson, B.Sc. HONS, PH.D.

Department of Medicine, University of Natal, Durban

V. Gathiram, M.B. CH.B., F.C.P. (S.A.), M.D.

Department of Medicine, Victoria Hospital, Wynberg, Cape Town

L. D. Regensberg, M.B. CH.B., M.R.C.P.

The Philippi farming area is situated 14 km from the centre of Cape Town on the edge of the Cape Flats (Fig. 1) and is virtually a rural island surrounded by suburban Cape Town. The 49 vegetable farms in the area cover approximately 5 000 hectares, most of which are under irrigation. The annual rainfall in Philippi is 700 mm and this water sinks rapidly through the sandy soil to join the Cape aquifer; this water can readily be recovered for irrigation as it accumulates on average 1,5 - 3 metres below the surface; agriculturally, therefore, Philippi is an excellent vegetable farming area.

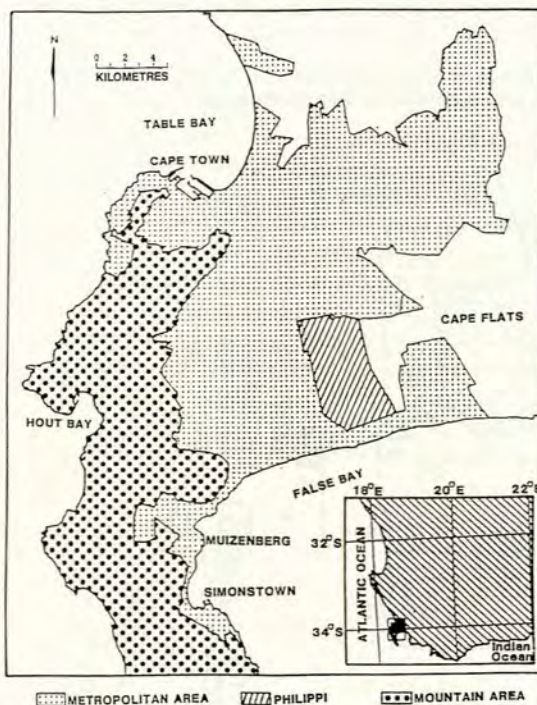


Fig. 1. Location of the Philippi farming area in the Cape Peninsula.

During the first half of 1984, doctors in the Department of Medicine at Victoria Hospital, Wynberg, noticed a marked increase in the number of patients from nearby Philippi with invasive amoebiasis (Fig. 2). Given the normally low prevalence of amoebiasis in Cape Town,¹ the matter was reported to the Medical Officer of Health of the Cape Divisional Council who initiated an investigation in collaboration with the Department of Community Health at the University of Cape Town.

Seven of the 9 patients admitted to Victoria Hospital came from one of the 49 farms (farm 40 in Fig. 3); the remaining 2 patients were resident on an adjacent farm (No. 44). The aim of the study was to determine whether the outbreak of amoebiasis was widespread or localised; the goal was to contain it, by detecting and treating infected subjects and by establishing and eliminating the source of the infection.

Entamoeba histolytica can be differentiated into pathogenic and non-pathogenic strains (zymodemes) by means of iso-enzyme electrophoresis of lysates prepared from amoebae isolated from *in vitro* culture; only pathogenic zymodemes have been isolated from patients with invasive amoebiasis, while asymptomatic carriers have been found to

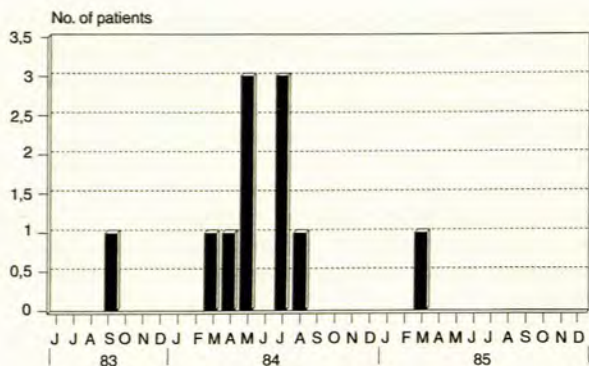


Fig. 2. Incidence of invasive amoebiasis among patients from the Philippi area admitted to Victoria Hospital.

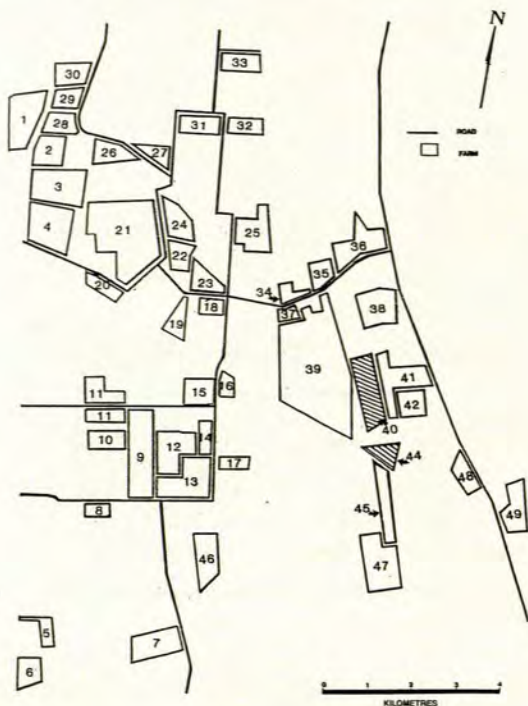


Fig. 3. The distribution of the farms in the Philippi area. The two shaded farms (40 and 44) are those from which the patients with invasive amoebiasis originated (farm 43 is not indicated because it had been incorporated by sale into another farm).

harbour both zymodeme types.^{2,3} Asymptomatic carriers of pathogenic zymodemes have a seropositive response equivalent to that seen in patients with invasive disease.⁴ It has been proposed that more accurate appraisal of the epidemiology of amoebiasis can be attained by complementary use of both serological methods and zymodeme analysis.⁴ Briefly, culturing of stools provides overall prevalence figures (both pathogenic and non-pathogenic zymodemes) while the serological response can be used to detect subjects infected with pathogenic zymodemes.^{4,5} This outbreak provided a unique opportunity to evaluate these proposals in terms of case finding, monitoring of the extent of endemicity and containment of disease transmission.

Materials and methods

The study population

In 1984, the farming community of Philippi totalled 1 998 people, and comprised white farmers, their families, coloured farm workers and their families. Fig. 3 details the location of farms in the area. There was a marked contrast between the quality of the housing and sanitation of the farmers and those of the workers; the farmers lived in modern housing incorporating comprehensive water reticulation, while the workers lived in overcrowded conditions usually without piped water or water-borne sewage. The investigation was carried out in 3 stages.

Stage 1 — detection and treatment of cyst-passers

The classic approach to the detection of carriers of *E. histolytica* was used, i.e. the examination of stool specimens for parasites at the cystic stage. A consulting epidemiologist recommended that at least 10% of the inhabitants on each of 20% of the farms in the area be sampled. Consequently, the two farms (40 and 44) from which patients were admitted to Victoria Hospital, as well as a further 8 randomly selected farms, were examined (Fig. 4).

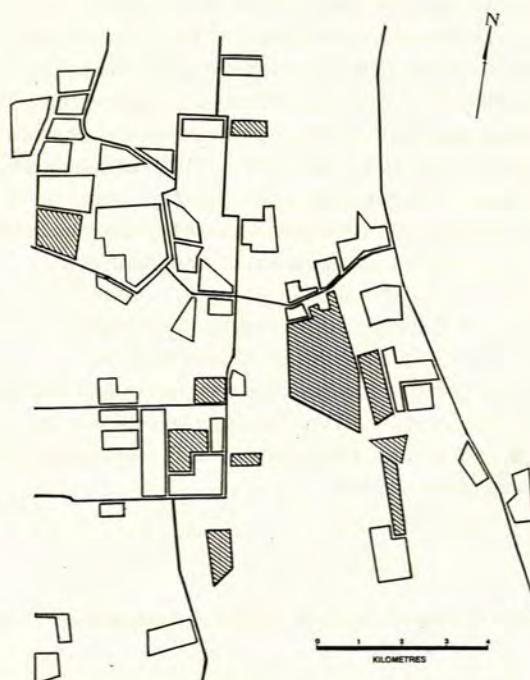


Fig. 4. The 10 farms sampled for the determination of the prevalence of *E. histolytica* cyst-passing in the Philippi farming area.

Everybody on farms 40 and 44 was treated. Additionally all inhabitants of any of the remaining farms where cyst-passers were detected were also treated.

Stage 2 — sero-epidemiology and zymodeme analysis

Personnel at the Medical Research Council (Natal), Durban, were consulted. They decided to expand the investigation to include serological investigations and zymodeme determination. The following farms were chosen for the study: (i) those on which patients who had been hospitalised for invasive amoebiasis had resided; (ii) farms adjacent to the above; (iii) farms on which cyst-passers of *E. histolytica* had been detected in stage 1; and (iv) all farms on which resided individuals who were either close friends or relatives of seropositive patients detected during stage 2. This decision was taken because previous work has shown that *E. histolytica* tends to cluster in related individuals.⁶

As a result the serological responses of 441 of 755 subjects on 16 of the 49 farms (Fig. 5) were assessed. Stools for *E. histolytica* culture and zymodeme determination were collected from 199 of 444 individuals from those 9 farms on which seropositive individuals were identified (Fig. 6).

All subjects who had antibodies to *E. histolytica* and who had not been treated in stage 1 were treated; close family members of these subjects were also treated. Previously treated individuals who were still infected with a pathogenic zymodeme were retreated. All people who had antibodies to *E. histolytica* were interviewed in order to determine their familial/social contacts in an attempt to establish possible infection pathways.

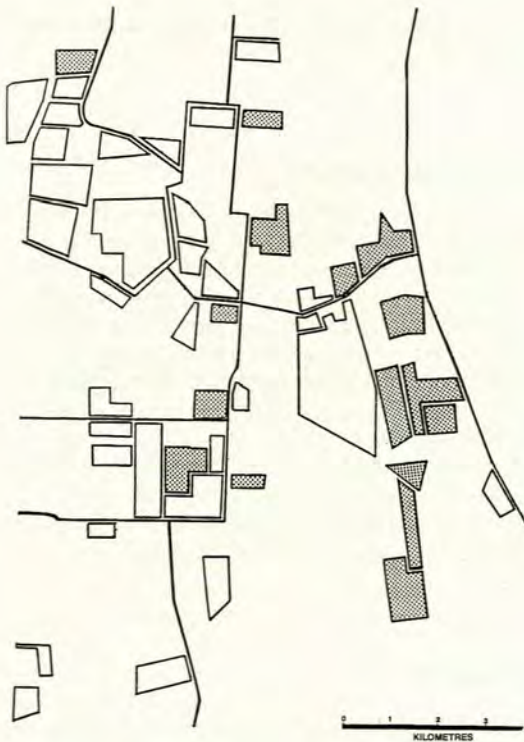


Fig. 5. The 16 farms sampled for the determination of *E. histolytica* serological responses.

Stage 3 — prospective and retrospective epidemiological surveys

A retrospective search of records at Victoria Hospital (June 1983 - February 1984) and a prospective one (September 1984 - December 1985) were conducted to determine prevalences of invasive amoebiasis originating from Phillippi before and after the outbreak of amoebiasis described in this article.

Blood was collected in June 1985 from 160 subjects on 4 of the 5 farms (40, 44, 45 and 47) found to have high prevalences of seropositive patients during our 1984 survey. The aim of this was to ascertain the level of endemicity following the mass treatment programme described above.

Treatment protocols

Inpatients with either amoebic dysentery (3 cases) or amoebic liver abscess (8 cases) were treated with metronidazole (Flagyl; Maybaker) 800 mg 3 times a day for 7 days; aspiration of the liver abscesses was performed in all but 1 patient. There were 2 fatalities — 1 elderly man died from an unrelated cause and the other died after rupture of his liver abscess into the pericardium. The remaining patients all recovered uneventfully.

Subjects described in stages 1 and 2 above were treated with metronidazole 800 mg 3 times a day for 7 days. The dispensing of the drug was supervised by the farmers. However, with this regimen there were occasional disulfiram-



Fig. 6. The farms where positive serological responses were recorded and from which stools were collected for *E. histolytica* culture and zymodeme determination.

like side-effects. Where this occurred treatment was changed to tinidazole (Fasigyn; Pfizer) 2 g daily for 3 days; this proved a simpler and more acceptable drug regimen.

Culture and zymodeme techniques

Amoebae were isolated from stools with Robinson's culture medium.⁷ Lysates were prepared from all *E. histolytica* isolated, and zymodemes determined by means of previously described techniques.²

Serological methods

Amoebic gel diffusion (AGDT) and indirect fluorescent antibody (IFAT) tests were performed as previously described;^{8,9} in the case of the IFAT only the specific IgG value was determined. An individual was judged serologically positive when one or both tests proved positive. A response in the AGDT was regarded as strongly positive when precipitins were seen at 20 hours and in the IFAT when the titre was greater than 1/500.

Results

Stage 1

Cyst-passers were detected on 4 of the 10 farms studied (Nos 32, 40, 44, 45); patients with proven invasive amoebiasis were residents of 2 of these farms (Nos 40, 44). Farm 32 is also of interest and is discussed below (stage 2).

Stage 2

Serological analysis revealed that the distribution of seropositive subjects was similar (Fig. 6) to that of the cyst-passers. Higher prevalences (> 10%) of seropositive responses occurred on the 2 farms (Nos 40, 44) where the hospitalised patients resided, as well as on 3 adjacent farms (Nos 38, 45, 47) (Table I). Furthermore it will be noted that on these farms 62,5 - 100% of the seropositive subjects had strongly positive serological findings. Isolations of pathogenic zymodemes were made on 4 of these farms (38, 40, 45 and 47). Weak to negative serological responses were found on the remaining farms (Fig. 6).

There were no subjects with antibodies to *E. histolytica* on farm 32 where cyst-passers had been detected in stage 1; this indicates that they were not infected with the pathogenic zymodeme of *E. histolytica*.

Table I. Farms on which seropositive subjects resided and the extent of the seropositivity

Farm No.	No. tested	Positive		Strong positive	
		No.	%	No.	%
12	73	2	2,7	0	
25	83	1	1,2	0	
30	22	3	13,6	0	
38	16	4	30,7	4	100
40	70	14	20	11	78,6
42	16	1	6,2	0	
44	39	8	20,5	5	62,5
45	36	5	13,9	4	80
47	45	6	13,3	4	66,6

One person who had apparently been treated during stage 1 was found to be infected with a pathogenic zymodeme; he later admitted that he had not taken the prescribed medication. Five other isolations of pathogenic zymodemes were made. All carriers of pathogenic zymodemes had strong seropositive responses. Eighteen isolations of non-pathogenic zymodemes were made; serological investigations were done on 14 of these and proved uniformly negative.

Stage 3

One further case of invasive amoebiasis was detected in the 15 months following the mass treatment programme (Fig. 2). Serological studies showed that of the seropositive subjects 72% were strongly seropositive in 1984 whereas only 11,5% were strongly positive in 1985 (Fig. 7).

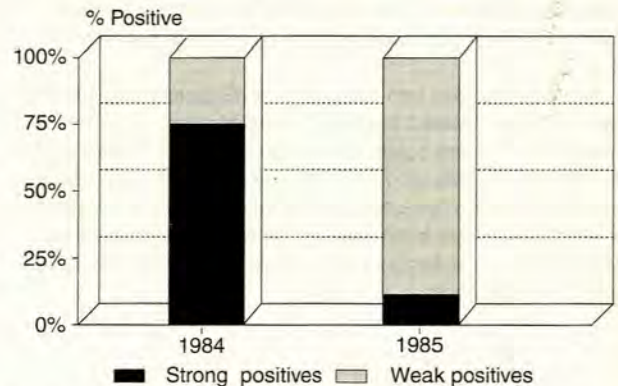


Fig. 7. Comparative serological results on farms 38, 40, 44, 45 and 47 in 1984 - 1985.

Mechanism of spread

The interviews with farm workers revealed that those who had antibodies to *E. histolytica* or who had had clinical disease were either relatives or friends and resided on the same or adjacent farms; they visited each other frequently and often ate and drank together. It is therefore postulated that *E. histolytica* was readily spread via the faecal-oral route. Residents of farms where seronegative responses were recorded proved to have had negligible contact with the seropositive subjects described above. The lack of personal or public transport in the area tended to make this a closed community, thereby confining the epidemic to a small area. The proposed infection pathways are depicted in Fig. 8. However, the source of the pathogen could not be established.

Discussion

This study has once again highlighted previous observations on the value of serology in detecting pathogenic zymodemes of *E. histolytica* in a population; carriers of pathogenic zymodemes tend to be seropositive while non-pathogenic zymodemes do not stimulate an antibody

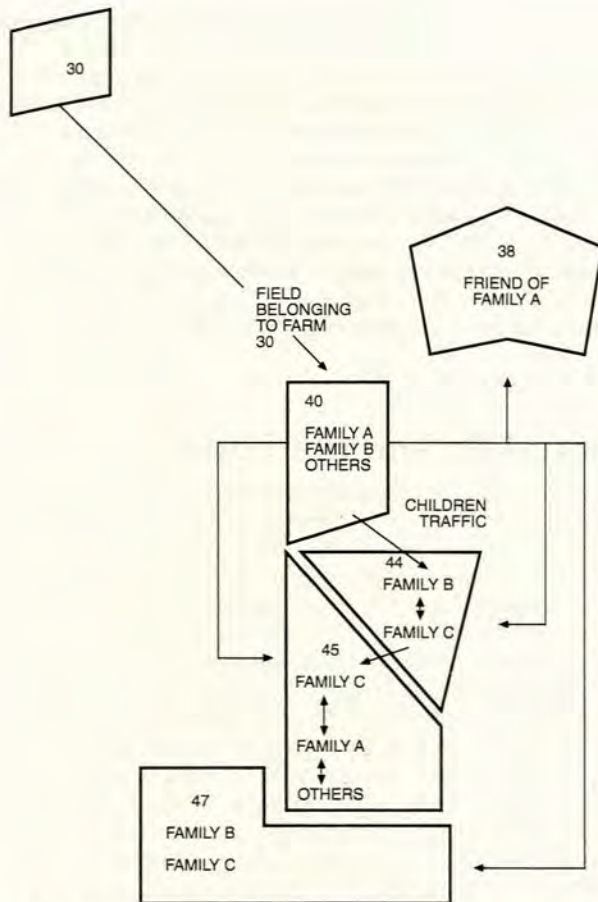


Fig. 8. Possible pattern of spread of pathogenic zymodemes of *E. histolytica* in the Philippi farming area.

response in their hosts.⁴ The clustering of infections implies faecal-oral transmission of the parasite and concurs with previous observations in Durban.⁵ In an epidemiological study of 2 cases of amoebic liver abscess in children, Harrison *et al.*¹⁰ found that in the family of 1 of them, 3 of 4 members had serological evidence of infection and 1 of 17 members of the family of the other child had positive serological findings while 8 were cyst-passers. Spencer *et al.*¹¹ conducted an investigation of the extended family of 2 cousins who developed amoebic liver abscess; they found that 49% of 162 family members had positive indirect haemagglutination tests for amoebiasis and 12,6% were passing cysts of *E. histolytica*; only 0,3% of a random sample of the remainder of the community was seropositive. From earlier work in Durban⁶ and the above studies^{10,11} it was concluded that investigation of the family or close contacts of patients with amoebiasis may disclose additional infected individuals who may be treated cost-effectively with minimal inconvenience. We believe that the dramatic reduction in the number of patients with invasive amoebiasis and the swing towards weak-positive to negative serology in the year following the mass treatment programme resulted directly from adoption of the above policy.

It has been proposed that the construction of antibody profiles may help in the prediction of the endemicity of

amoebiasis in a region.⁴ In the amoebiasis-endemic area of Durban a bimodal distribution of antibody titres was observed with the majority of seropositive subjects having weakly positive serology indicative of past contact with pathogenic zymodemes of *E. histolytica*.⁴ An epidemic is indicated when the majority of seropositive subjects have strong responses as observed in stage 2 of the present study. Consequently the efficacy of a treatment strategy for containment of an epidemic can be monitored by observing a swing in the antibody profile from that of the typical epidemic to that of an endemic pattern (Fig. 7).

Although *E. histolytica* does occur in this area, the low prevalence of seropositivity in a previous serological survey¹ indicates that infections with non-pathogenic zymodemes predominate. The infection pathways constructed during the present study implied that the outbreak occurred as a result of the introduction of a pathogenic zymodeme into this previously non-immune population. Mirelman *et al.*¹² reported that they had managed to convert a non-pathogenic into a pathogenic zymodeme of *E. histolytica in vitro* by varying the culture conditions; on the basis of this observation they claim that invasive disease results from the *in vivo* conversion of a non-pathogenic to a pathogenic organism. The results of the present study refute this hypothesis since transmission of infection, in this community, by the pathogenic zymodeme could be accurately delineated. This suggests that the invasive potential of pathogenic zymodemes of *E. histolytica* in their natural host is an immutable stable feature.¹³

The technical expertise of Mrs C. B. Anderson and Mr S. Suparsad was invaluable. We acknowledge the assistance of Mr Gys White of the Western Cape Regional Services Council and the support of this Council. We thank the South African Medical Research Council for their financial support.

REFERENCES

- Maddison SE, Powell SJ, Eidsdon-Dew R. Application of serology to the epidemiology of amoebiasis. *Am J Trop Med Hyg* 1965; **14**: 554-558.
- Sargeant PG, Williams JE, Greene JD. The differentiation of invasive and non-invasive *Entamoeba histolytica* by isoenzyme electrophoresis. *Trans R Soc Trop Med Hyg* 1978; **72**: 519-521.
- Sargeant PG, Jackson TFHG, Simjee AE. Biochemical homogeneity of *Entamoeba histolytica* isolates especially from liver abscess. *Lancet* 1982; **1**: 1386-1388.
- Jackson TFHG, Gathiram V, Simjee AE. Seroepidemiological study of antibody response to the zymodemes of *Entamoeba histolytica*. *Lancet* 1985; **1**: 716-718.
- Gathiram V, Jackson TFHG. Frequency distribution of *Entamoeba histolytica* zymodemes in a rural South African population. *Lancet* 1985; **1**: 719-725.
- Gathiram V, Jackson TFHG. A longitudinal study of asymptomatic carriers of pathogenic zymodemes of *Entamoeba histolytica*. *S Afr Med J* 1987; **72**: 669-672.
- Robinson GL. The laboratory diagnosis of human parasitic amoebae. *Trans R Soc Trop Med Hyg* 1968; **62**: 282-294.
- Jackson TFHG, Duursma J, Simjee AE, Pudifin DJ. Application of the leucocyte adherence inhibition test to the assessment of cell mediated immunity in invasive amoebiasis. *Trans R Soc Trop Med Hyg* 1983; **77**: 121-125.
- Jackson TFHG, Anderson CB, Simjee AE. Serological differentiation between past and present infection in hepatic amoebiasis. *Trans R Soc Trop Med Hyg* 1984; **78**: 342-345.
- Harrison HR, Grave CP, Fulginiti UA. Amoebic liver abscess in children: clinical and epidemiological features. *Pediatrics* 1979; **64**: 923-928.
- Spencer HC, Muchnick C, Sexton DJ, Dodson P, Walls K. Endemic amoebiasis in an extended family. *Am J Trop Med Hyg* 1977; **26**: 628-634.
- Mirelman D, Bracha R, Wexler A, Chayen A. Changes in isoenzyme patterns of a cloned culture of non-pathogenic *Entamoeba histolytica* during axenisation. *Infect Immun* 1986; **54**: 827-832.
- Gathiram V, Jackson TFHG. Pathogenic zymodemes of *Entamoeba histolytica* remain unchanged throughout their life-cycle. *Trans R Soc Trop Med Hyg* 1990; **84**: 806-807.

Accepted 24 Mar 1993.