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patrones isoenzimáticos de cepas de *Entamoeba histolytica* aisladas en Columbia Británica*

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isoenzyme patterns of strains of *Entamoeba histolytica* isolated in British Columbia*

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Resumen

Se determinaron de manera electroforética los patrones isoenzimáticos de 86 aislamientos de *Entamoeba histolytica*. La sintomatología de los individuos varió entre dolor abdominal con estreñimiento leve y diarrea con sangre y moco. Se valoró la seropositividad tanto por la prueba de difusión amibiana en gel (AGD) como por la prueba inmunosorbente ligada a enzima (ELISA). Sólo siete de los lotes establecidos en cultivo resultaron zimodemos patógenos.

Abstract

The isoenzyme patterns of 86 isolates of *Entamoeba histolytica* were electrophoretically determined. Symptomatology of the individuals varied from abdominal pain with mild constipation to diarrhea with blood and mucus. Seropositivity was assessed by both the amoebic gel diffusion test (AGD) and the enzyme linked immunosorbent assay (ELISA). Only seven of the stocks established in culture proved to be pathogenic zymodemes.

Entamoeba histolytica has a cosmopolitan distribution and is estimated to affect about 10 per cent of the world population. The prevalence of infection varies with the level of sanitation and is generally higher in tropical and subtropical countries. Aside from climate and social conditions, factors inherent in the amoebae are thought to play a part in the production of disease. Whilst the incidence in Canada, outside institutionalized groups, is less than one per cent, endemic foci have been shown to exist.[1] Although disease manifestations in British Columbia are rare, there has been a fourfold increase in the past few years in the number of specimens identified by the Provincial Public Health Laboratory as harbouring *Entamoeba histolytica*. This may be the result of increased travel by British Columbians to Central and South America, the Pacific Rim and Asian countries where they are at considerable risk of acquiring infection with intestinal protozoa. It may also be attributed to an increased awareness of amoebiasis in the homosexual population in Canada and the U.S.A.[2,3]

The definitive diagnosis of infection with *Entamoeba histolytica* depends upon the demonstration of the organisms in faecal material. Microscopic identification of this parasite and its differentiation from other species of amoebae requires considerable skill. In addition, the degree of virulence or pathogenicity of a particular isolate, is not a demonstrable characteristic. The application, in recent years, of thin-layer starch-gel electrophoresis to the study of stocks of *Entamoeba histolytica* has permitted the differentiation on the basis of isoenzyme patterns bertween species and between pathogenic and non-pathogenic strains.[4,5] Studies to date in Mexico, New Delhi, United Kingdom and South Africa, have characterized 20 isoenzyme patterns, eight of which have originated from cases of clinical amoebiasis and are considered to be pathogenic zymodemes.[6-8]

The aim of the study was to characterize all strains of *Entamoeba histolytica* isolated in British Columbia, with a view to possible change in the public health policy and re-evaluation of the present therapeutic considerations.

Materials and methods

When *Entamoeba histolytica* was diagnosed microscopically in stools referred to the Provincial Public Health Laboratory in SAF preservative, unpreserved specimens for cultivation of amoebae were then requested. The isolation of *Entamoeba histolytica* was achieved by inoculation of faecal material into Robinson's medium.[9] Lysates and permanent slides stained by iron haematoxylin were prepared from each strain established in culture. The lysates were employed to determine the electrophoretic isoenzyme patterns and the stained slides for microscopic confirmation of the presence of *Entamoeba histolytica*. The techniques employed were those described by Sargeaunt and Williams.[4] The isolates were characterized by the electrophoretic patterns of four enzymes: EC 5.3.1.9. glucosephosphate isomerase (GPI); EC 1.1.1.40., L-malate: $NADP^+$ oxidoreductase (oxaloacetate decarboxylating) (ME); EC 2.7.5.1., phosphoglucomutase (PGM); and EC 2.7.1.1., hexokinase (HK). *Escherichia coli,* the bacterial associate, which served as a control, was grown in Robinson's medium. For purposes of this study, the cases were divided into male homosexuals and male and female heterosexuals. All infected individuals were requested to complemete a questionnaire regarding travel history, symptomatology ans sexual practices. Serum samples were tested for the presence of antibodies to *Entamoeba histolytica* by means of the amoebic gel diffusion precipitin test (AGD)[10] and the enzyme linked immunosorbent assay (ELISA).[11]

Results

Only 86 (43 per cent) isolates were established in culture from the 200 patient specimens which were identified microscopically as having *Entamoeba histolytica*. The distribution of the zymodemes and the serological findings are shown in table I. The isoenzyme patterns of interest to this study are diagramatically represented in figure 1. Only five of the zymodemes, I, II, III, XIV and XVII, described by Sargeaunt and coworkers were identified. Non-pathogenic zymodemes I and III were the most common; zymodeme I, being identified more frequently than zymodeme III. Twenty-nine of the heterosexual males and females with non-pathogenic zymodemes had a history of travel compared with only six of the homosexual males. Six of the seven individuals with pathogenic zymodemes had travelled outside of North America.

The absence of an α band and the presence of a β band in PGM, along with advanced bands in HK is

TABLE 1 DISTRIBUTION OF ZYMODEMES AND SEROLOGICAL FINDINGS

Category	Number	Clinical Manifestations	Zymodemes I	II	III	XIV	XVII
		S	8	4	3	1	1
Heterosexuals	45						
		A	21		5	1	1
		S	14		6		1
Homosexuals	27						
		A	4		2		
Uncompleted Questionnaire	14		10	1	3		
Total	86		57	5	19	2	3
Serology: Number positive/ Number tested.	4/21		0/11	1/3	1/4	2/2	0/1

S = Symptomatic
A = Asymptomatic

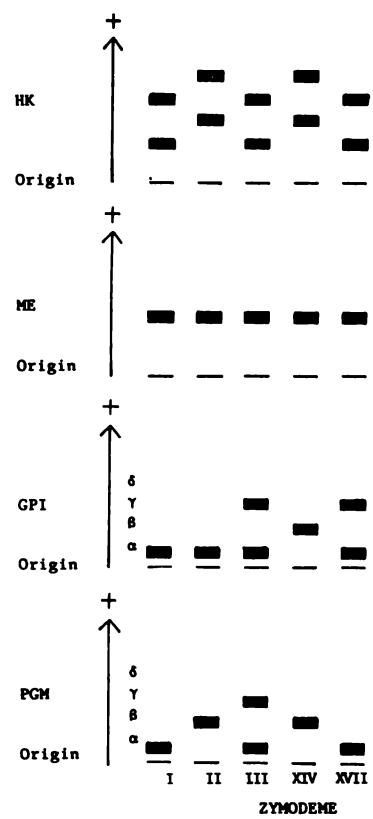

Figura 1. Diagrammatic representation of the isoenzyme patterns.

considered to be a pathogenic marker. An δ band alone or paired with either ϱ or s in GPI is found in all strains, while a single band in ME is characteristic of *Entamoeba histolytica*.[4] Serum was obtained from only five of the seven individuals who were identified with enzyme patterns characteristic of pathogenic zymodemes (II, XIV). Of these individuals, three (60 per cent) were serologically positive compared with only 6 per cent of the individuals from whom non-pathogenic zymodemes were isolated.

Symptoms varied from diarrhea with blood and mucus, alternating with intermittent constipation in those who harboured pathogenic *Entamoeba histolytica*, to mild abdominal discomfort in those with non-pathogenic *Entamoeba histolytica*.

Only 26 per cent of the heterosexuals harbouring non-pathogenic zymodemes were symptomatic compared with 77 per cent of male homosexuals. Five of the six individuals harbouring pathogenic zymodemes were symptomatic. The seventh individual from whom a pathogenic zymodeme was isolated did not complete the questionnaire. Twenty heterosexual individuals and seven homosexual men harboured only *Entamoeba histolytica*, whereas the remainder of the individuals were infected with more than one parasite (table 2). Eight heterosexual individuals and five homosexual males had concomitant *Giardia lamblia* infections. Helminths were identified in only two individuals, both of whom had travelled. Nineteen of the individuals tested were females. The mean age of the heterosexuals was 34 (16-73), whereas the homosexuals had a mean age of 35 (26-54).

Discussion

Two previous reports from the North American continent were concerned with the evaluation of a PAGE isoenzyme system [12] and the assessment of virulence of pathogenic and non-pathogenic strains of *Entamoeba histolytica* in an animal model.[13] In this, the first report of the isoenzyme patterns of strains of *Entamoeba histolytica* from British Columbia, the number of isolates studied far exceeded those of earlier reports.

The male homosexuals were considered as a separate group in the analysis of the data in this study because they frequently present with multiple intestinal infections, a condition referred to as "Gay Bowel Syndrome", and because of the increased incidence of *Entamoeba histolytica* in this group in recent years. In accordance with Sargeaunt and coworkers's findings, no pathogenic zymodemes were expressed by strains of *Entamoeba histolytica* isolated from patients identified as male homosexuals.[14] All the stocks belonged to zymodemes I and III, the isoenzymes which have been most commonly encountered in surveys throughout the world. The number of male homosexuals who were symptomatic, although they were harbouring *Entamoeba histolytica* of a non-pathogenic zymodeme, was remarkably high. Since examination of a single stool specimen does not exclude the presence of other intestinal parasites and, as bacterial cultures were not performed, it may be that the symptoms, both within the male homosexual group and the other study cases, were due to other enteric pathogens. In those with concomitant infections with *Giardia lamblia*, symptoms may well have been attributable to this organism.

This is the first time that both the potentially pathogenic zymodeme XIV and the non-pathogenic zymodeme XVII have been isolated from individuals on the North American continent. Neither of the two cases from whom stocks of the pathogenic zymodeme XIV were isolated presented with clinical manifestations associated with amoebiasis, a feature which

TABLA 2
PROTOZOAN PARASITES HARBOURED BY THE 86 INDIVIDUALS FROM WHOM *Entamoeba histolytica* WAS CULTIVATED

	Heterosexuals	Homosexuals	Uncompleted Questionnaire
Entamoeba histolytica only	20	7	6
E. histolytica + 1 Parasite	11	9	3
E. histolytica + Parasites	7	6	4
E. histolytica + 3 or more parasites	7	5	1

Sargeaunt et al[8] observed in some of the patients in whom this zymodeme was identified in India. Previous studies have suggested that zymodeme XIV is peculiar to the Indian subcontinent, yet only one of the two individuals with this isoenzyme had travelled to India. The patient had a negative AGD, an ELISA of 1:1024 and a concomitant Giardia infection. The other patient had only travelled to the Philippines and, although considered to be clinically well, had a positive AGD and an ELISA of 1:512. It was not possible to follow either of these patients to determine whether symptoms subsequently developed. Two of the individuals identified as harbouring *Entamoeba histolytica* of pathogenic zymodeme II, were serologically negative. Clinical data were not available on one of the individuals and the other probably acquired his infection in Canada. In the past ten years, the only travel history was a visit, approximately six months prior to hospitalization, to an Indian Reserve in Alberta, Canada, one of the provinces where endemic foci of *Entamoeba histolytica* are known to exist. Laboratory examination revealed haematophagous trophozoites of *Entamoeba histolytica*, yet both the AGD and ELISA were negative. The patient was concomitantly infected with *Giardia lamblia* and diagnosed as being hypogammaglobulinemic, a condition which may have accounted for his negative serology. One of the heterosexuals from whom a non-pathogenic zymodeme was isolated was serologically positive, suggesting a previous infection with a pathogenic strain since antibodies are only produced in response to tissue invasion and may persist for long periods post therapy. Further investigations revealed that the individual had been treated in the course of his travels, six months previously, for amoedic. dysentery. Both the ELISA and AGD were positive, a finding consistent with a recent infection.

Correlation between the initial microscopic diagnosis and subsequent electrophoretic findings was excellent. Where *Entamoeba histolytica* was identified microscopically, the pattern of the malic enzyme confirmed the species. Specimens with mixed infections of *Entamoeba histolytica* and other protozoans were common (tabe 2) and accounted for 62 per cent of the total specimens; yet in every instance, *Entamoeba histolytica* outgrew the other organisms. As only 43 per cent of known positive specimens were established in culture, it would seem that conventional microscopy is a more effective method of locating the parasite than cultivation. This applies particularly where there is a delay between evacuation of a specimen and inoculation of culture medium. Whilst the electrophoretic determination of isoenzyme patterns is too costly and complicated a technique to be implemented as a routine laboratory method, it does have a place in a reference or referral centre.

Only seven of the 86 stocks established in culture proved to be pathogenic zymodemes and, in all probability, only one of the individuals acquired the infection within the country. The remaining 79 stocks were non-pathogenic. On the basis of the zymodemes, irrespective of the clinical manifestations, it is apparent that the vast majority of the individuals in British Columbia habour non-pathogenic strains. The current therapy for amoebiasis entails administration of a lumen acting drug for symptomless carriers and a combination of lumen acting and tissue acting drug for symptomatic individuals. In light of the present findings which suggest that 79 individuals may have been treated unnecessarily with potentially toxic drugs, recommendations will be made that consideration be given to present public health policy which requires that all individuals harbouring *Entamoeba histolytica* be treated irrespective of the zymodeme of the isolate and whether or not they are symptomatic.

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