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The epidemiology of infection with *Entamoeba histolytica* in The Gambia, West Africa

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Summary

A country-wide stool survey of The Gambia showed the prevalence of *Entamoeba histolytica* cysts to range from 13·7% up-country in the dry season to 52·3% near the coast. A longitudinal survey showed a near 100% infection rate over one year and a sharp rise in prevalence as the rains commence with an equally sharp fall as the rains progress. Specific antibody levels are elevated and reasonably constant through the year.

Carriers generally show no specific lymphocyte reactivity to amoebic antigen but consistently parasite-negative individuals tended to show elevated lymphocyte reactivity.

Attempts to discover the presence of cysts in the environment of villages by cultivation of specimens of water, soil, food, flies and washings from clothes and hands generally failed though *E. histolytica* was recovered once from a well.

Introduction

The epidemiology of amoebiasis has been reviewed in recent years by ELSDON-DEW (1968), ANDRÉ (1971) and BRAN (1974) but little has been added in that time to our knowledge of the subject. STAMM's (1970) aphorism "it is unknown by which of the possible pathways of transmission cysts are most commonly carried from faeces to mouth" remains true. The difference of opinion between SAPERO & JOHNSON (1939) and SCHOENLEBER (1940) about the importance of the food handler remains unresolved. The only certainty appears to be that focal amoebiasis appears if cross-connection between faecal waste and drinking water supplies occurs.

The immune response of the host is also central to the epidemiology of amoebiasis and we would contend (BRAY & HARRIS, 1977) with KAGAN (1974) central too to the pathogenesis of invasive amoebiasis. Little attention however has been paid to the specific immunological responses, especially cellular responses of whole populations at risk where the early events in the immune response may control establishment and invasiveness of the organism.

Epidemiological surveys revealed a comparatively high incidence of infection with *E. histolytica* in The Gambia and seemed to provide a fertile area for research into pathways of transmission and the immunological parameters which may govern the incidence of both infection and disease.

Materials and methods

The environment

The Gambia is a typical West African grassland savannah region, with some lightly forested regions on the ironstone plateau inland. The climate is highly seasonal with a hot, wet season from mid-June to mid-November, and somewhat cooler, dry weather for the remainder of the year. Most of the population are peasant farmers living in small, more or less isolated villages. In contrast, parts of the coastal plain are becoming increasingly urbanized and overcrowded.

The epidemiological study comprised three main projects. Firstly, a nationwide survey was carried out to establish the overall incidence and intensity of infection with *E. histolytica*, and to identify any areas of particular prevalence. 26 villages, covering all geographical regions of the country, were visited during the dry season March–April 1975. 50 individuals of mixed sex and age were selected from each village, and a single stool sample and a caraway tube of blood was collected from each. Stools were examined in a field laboratory by formol-ether concentration, and the presence of all intestinal parasites recorded. Serum was prepared from the caraway tubes, frozen at −20°C in the absence of preservative, and transported in ice to the main laboratories at Fajara for further examination.

Selected villages were revisited during the wet season August 1975 and repeat samples collected in order to determine the change in infection and antibody levels with season.

Secondly, a detailed longitudinal study was initiated in January 1974 of four small villages with a total population of about 150 people, mostly of the Manjago tribe, situated approximately one hour's drive south of the main laboratories.

Sanitation in these villages is non-existent with defaecation occurring at random in the bush around the village. Water is drawn from wells situated in, or close to, the living compounds, and is used directly for drinking, cooking and washing, or stored in pots for later use.

A complete census of each village was taken, and a stool sample collected each month from, as far as possible, everybody on the original census. Normal formed stools were examined by formol-ether concentration, liquid or bloody stools were examined by direct microscopy and by culture in Robinson's medium (ROBINSON, 1968), as well as by concentration. Every three months, a single caraway tube of blood was taken from each person, and the serum prepared and stored at −20°C for further study. Blood films for malaria and blood for haematocrit and haemoglobin estimation were also collected.

A single village of 370 people, situated approximately 45 miles east of Fajara, was added to the longitudinal survey in November 1975, to compensate for a slow migratory loss of the Manjago tribesmen. The population of this village was primarily Mandinka, with a few Jola and Manjago families. Living conditions were similar to those described above. A complete census was taken and samples were collected as above.

Thirdly, efforts were made to identify the pathways whereby transmission of cysts was occurring in the village environment. From the longitudinal study

villages, the following materials were collected periodically throughout the year: 5 or 10-litre lots of water from wells or storage pots, hand washings in distilled water and saline, clipped fingernails in distilled water and saline, soiled clothing, house-flies, lettuce purchased in the local market, and soil collected from around the living compounds (in the rainy season). Water and the washings from hands, finger-nails, clothing and lettuce were either centrifuged at 500 g for 10 minutes and the deposit collected, or filtered through millipore filters of 4·5 mc pore size. The particulate matter was divided into two aliquots; one half was cultured in Robinson's medium (ROBINSON, 1968) or in HSre slopes plus rice starch (DOBELL & LAIDLAW, 1926) and subcultured twice at 48-hour intervals, the other half was examined by direct microscopy. Flies were either allowed to walk on the agar slopes of Robinson's medium and then the liquid phase added or they were allowed to walk, defaecate and regurgitate on to inspissated serum slopes and then the Ringer's albumin rice-starch overlay added.

Serology

Specific circulating anti-amoebic whole immunoglobulin and, in certain cases, IgM, G and A were measured by the indirect fluorescent antibody method (IFA) of KANE, MATOSSIAN & BATTY (1971), using antigen prepared locally from the NIH:200 strain of *E. histolytica* or purchased from Wellcome Reagents Ltd., Beckenham, England. Some stools containing high IgA levels were examined for anti-amoebic IgA by IFA using tagged anti-monomeric IgA.

Lymphocyte transformation

Peripheral blood was collected by venepuncture from selected villagers (or patients), and lymphocytes concentrated and cultured with amoebic antigen or phytohaemagglutinin (PHA) as previously described (HARRIS & BRAY, 1976). Counts per minute were transformed to $\log_{10}$ for statistical analysis.

Results

I. *National survey*

Fig. 1 is a map of The Gambia, divided into its geographical regions, with the figures for percentage prevalence of *E. histolytica* calculated from the samples collected during the dry season (March–May) 1975. Table I gives the percentage prevalence over the country by sex and age as well as by geographical region.

For the dry season the over-all incidence of infection in the Upper River Division (URD) was 13·7% of adults, of which the majority were females; 18·30% of females were passing cysts, as against 8·43% of males. Moving westwards, in the McCarthy Island Division (MID) an over-all incidence of 22·7% was recorded, North Bank Division (NBD) was 56·2%, Lower River Division

Table I – Percentage prevalence by age, sex and geographical area of infection with *Entamoeba histolytica*

	Age distribution				Sex distribution	
	0–2 yrs	3–10 yrs	11–14 yrs	15+ yrs	male	female
URD North	0·0	11·8	12·5	17·4	8·3	20·8
South	14·3	10·6	14·3	10·7	8·5	15·8
Over-all	12·5	10·9	13·9	13·7	8·4	18·3
MID North	11·1	12·8	9·5	25·0	22·6	4·5
South	0·0	10·9	5·9	21·8	11·8	16·0
Over-all	6·7	11·7	7·9	22·7	17·2	10·3
NBD	0·0	22·0	48·6	56·2	35·9	46·3
LRD	0·0	28·0	42·9	29·1	25·4	30·0
WD	16·7	45·9	69·2	52·3	47·9	51·0

URD: Upper River Division.
MID: McCarthy Island Division.
NBD: North Bank Division.
LRD: Lower River Division.
WD: Western Division.

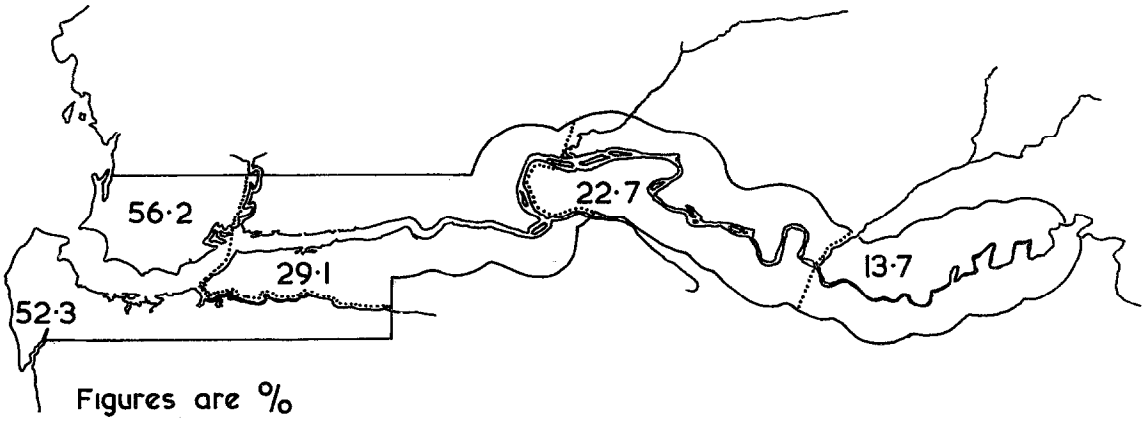

Fig. 1. Map of The Gambia showing over-all percentage prevalence of *Entamoeba histolytica* amongst the adult population in the various geographical regions.

(LRD) was 29·1% and the Western Division (WD), excluding Banjul, was 52·3%. So, in general, there was an increase in the percentage prevalence of infection moving east to west; this is associated with both the increasing population density and the generally higher humidity and slightly cooler temperatures, at least during the dry season, of the coastal plain. Living conditions and patterns of behaviour amongst the peasant farmers change very little with geographical region.

Interestingly, though, in Banjul—the capital city—an examination of 200 stools taken from a cross-section of townspeople living in overcrowded, highly insanitary conditions yielded only 15·5% positives. Transmission rates should be high under these conditions but this does not seem to be reflected in a raised infection level. We had also anticipated a comparatively high incidence of clinical amoebiasis in the town, which again did not materialize.

A further interesting feature of this cross-section was the sex differential; over-all 25·9% (116/447) of males were passing cysts, as against 45·2% (217/480) of females. This difference was particularly marked in the URD. Taking all our figures (i.e. including the longitudinal surveys) the figures are 22·8% males and 29·4% females passing cysts of *E. histolytica*. This may be compared with 36·9% for males and 48·9% for females passing cysts of *E. coli*, and 13·8% of males and 20·2% of females passing cysts of *Endolimax nana*, while slightly more males than females passed ova of hookworms. The wet season national survey showed an over-all increase of percentage prevalence in the URD and MID to 44·61% positives, but a decrease in the NBD to 24·15%.

II. *Longitudinal Survey*

Fig. 2 shows the change in percentage incidence of *E. histolytica* in monthly stool samples collected from the longitudinal study population over the seasons January 1974 to August 1976. From January to mid-June (the dry season), there was a steady decline in positives down to approximately 15% on a single stool sample.

The wet season in each year was marked by a sharp peak in the number of positive samples. Cysts will survive for much longer during the rains, and this peak may reflect a true increase in the number of new infections in the population. Alternatively, it may have been due to an increase in the demonstrability of the parasite, presumably by an increase in cyst production. Annual rhythms have been described for a number of parasites, and such a rhythm could be operating here.

Perhaps the most interesting part of this graph was the dramatic fall-off in the number of stools positive for four-nucleate cysts during the second half of the rains—a time when one would expect transmission to be increasing or at a maximum. The same rise and fall was seen in the case of infection with *E. coli* and *Endolimax nana*. Over-all and taking only those on whom 12 or more observations were made, 98·3% passed *Entamoeba hystolytica* cysts at some time, 100% passed cysts of *E. coli*, 94·9% passed cysts of *Endolimax nana*, 84·7% passed cysts of *Iodamoeba buetschlii* and 66·1% passed cysts of *Giardia*.

Table II shows the age distribution for cyst passers of five protozoan parasites using all surveys done. As can be seen *Entamoeba histolytica* and *E. coli* infections continue to be acquired throughout life but *Giardia* infection

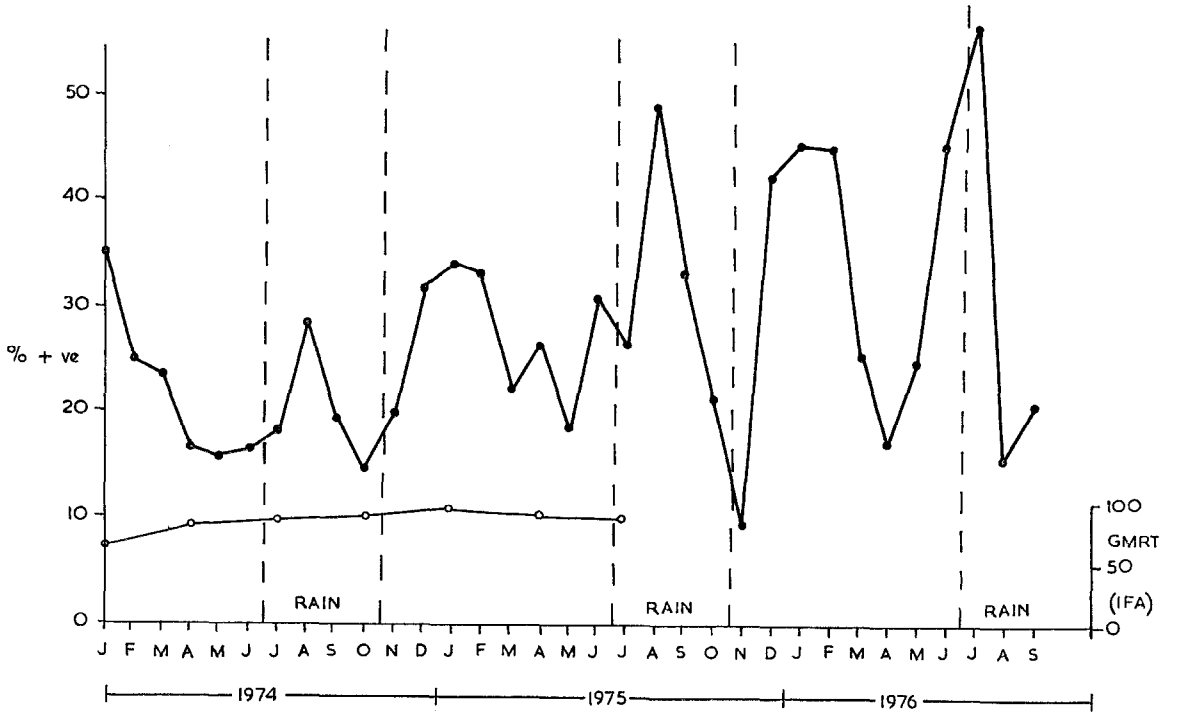

Fig. 2. Longitudinal change with time of over-all percentage prevalence of *Entamoeba histolytica* and of geometric mean reciprocal titre (indirect fluorescent antibody) for a long-term study population in the Western Division.

Table II – Percentage prevalence of five species of cyst-forming protozoa and hookworms (all surveys) by age. E.h. = *E. histolytica*, **E.c.** = *E. coli*, **E.n.** = *Endolimax nana*, **I.b.** = *Iodamoeba buetschlii*, **G** = *Giardia*, **Hw** = **Hookworm**

Age	E.h.	E.c.	E.n.	I.b.	G.	Hw.
0–12 m.	1·9	4·9	0·0	1·0	1·0	4·9
1–2 yr	6·8	11·0	7·6	1·7	11·4	7·2
3–4 yr	18·7	39·9	14·4	11·2	24·4	19·8
5–9 yr	22·7	44·2	15·9	7·9	21·4	22·7
10–14 yr	26·1	44·9	14·1	11·0	13·8	26·4
15–24 yr	31·1	52·1	19·2	16·5	8·5	24·7
25–39 yr	34·2	52·5	19·8	9·7	6·4	24·2
40+ yr	35·0	55·5	21·1	10·1	5·6	32·2
Total	939/3676	1581/3676	577/3676	335/3676	454/3676	826/3676
	25·5	43·0	15·7	9·1	12·4	22·5

peaks in the three to four-year-old age group.

Results of total specific anti-amoebic antibody determination by indirect fluorescence are given in Fig. 1 as the geometric mean reciprocal titre (GMRT) of the population at each quarterly bleed. At the whole population level, antibody titres were raised and more or less constant over the full year. Only occasional specific anti-amoebic IgA was detected in stools from the longitudinal study. No correlation between the presence of cysts and malaria, PCV or haemoglobin levels was detected.

III. *Route of transmission*

From all the different samples collected over the course of the past two years, we have been able to grow only one amoeba that was morphologically identical with *E. hystolytica* in stained films and that was from a sample of well water. This strain did not grow well *in vitro* and we were not able to apply critical tests such as temperature tolerance, enzyme variant specificity or DNA buoyant density determinations in order to type the organism.

Microscopically we were able to identify cysts of *E. coli* and ova of *Necator* in washings from clothing. From the culture samples, we have been able to isolate amoebae and flagellates from well water, which grow well at 22°C as well as coliform bacilli and a ciliate from stored water.

Lymphocyte transformation

Preliminary studies (HARRIS & BRAY, 1976) suggested that consistent absence of demonstrable infection was associated with a positive lymphocyte response to amoebic antigen *in vitro*, whereas lymphocytes from asymptomatic carriers as a group did not respond to amoebic antigen *in vitro*.

From our increased study population, we were able to identify a further five individuals (out of a group of 370) who were negative for four-nucleate cysts on repeated stool examinations over several months.

The mean $\log_{10}$ transformation index for this group (i.e. $\log_{10}$ cpm with antigen/$\log_{10}$ without antigen) was a positive value of 1·1405, whereas the same figure for an equal number of asymptomatic cyst passers was 1·0668. These results are not conclusive. The bleeds were taken during the wet season 1976 (August) and the population is subject to a multitude of assaults at this time, notably hyperendemic malaria (*Plasmodium falciparum*), that could substantially confuse the immune response to any one particular antigen.

Discussion

Studies on the host's immune response in amoebiasis have centred largely on responses in clinical disease, and a wide range of antibodies have been described in both intestinal and hepatic amoebic disease (MADDISON, KAGAN & NORMAN, 1968; KRUPP, 1970). Studies of cellular sensitivity in man appear to be limited to two reports on the sensitivity of lymphocytes from patients with liver abscess to amoebic antigen and mitogens in macroculture (SAVANAT, VIRIYANOND & NIMITMONGKOL, 1973; ORTIZ-ORTIZ, ZAMACONA-RAVELO & CAPIN, 1974). 21 of the 23 patients showed positive transformation, the cells producing between 31% and 23% of the response stimulated by PHA in one study. No correlation was detected between degree of blast transformation and humoral antibody response. In the other study of 11 patients with hepatic abscess only four reacted significantly to amoebic antigen.

The immune response in overt amoebic disease is only one aspect. Perhaps more important is the immune status and response at the time of initial invasion or attempted invasion. Whatever factors control the switch from commensal to aggressive form, the success of the aggressive parasite will depend on its ability to overcome the response of the host. Therefore, an examination of immune parameters at all stages of amoebiasis—ideally before, during and after invasion and particularly immediately prior to and during the invasive process—may yield important information on why *E. histolytica* does or does not successfully invade.

There is accumulating evidence to suggest that cell-mediated immunity is of major importance in determining the success or otherwise of potentially-invasive *E. histolytica* (SAVANAT, 1968; KAGAN, 1973), and the extent of the pathology produced (KAGAN, 1974).

Intestinal and liver lesions show a marked absence of cellular infiltration (BRANDT & PEREZ-TAMAYO, 1970), suggesting that local or central immunodepression is a prerequisite for successful invasion; although the work of MAEGRAITH & HARINASUTA (1954a, b) has suggested that sensitization with amoebic antigens, normally from the infected bowel, may be a requirement for the development of liver abscess.

Recovery from acute liver or intestinal amoebiasis is not always associated with the development of protective immunity (KNIGHT, SCHULTZ, HOSKINS & MARSDEN, 1973; JENKINSON & HARGROVE, 1975). Conversely, epidemiological evidence suggests that the immune response is important in limiting the invasive success of *E. histolytica* (KAGAN, 1974; KNIGHT, 1975), and that amoebic disease must be preceded by, and associated with, a greater or lesser degree of immunodepression.

Our results support, and to some extent explain, this apparent paradox. Within the population studied, infection rates are high (approaching 100% per annum in certain areas), and levels of anti-amoebic antibody are, on a population basis, raised and approximately constant with time. Within the population, that group which had at no time passed cysts of *E. histolytica* showed a significant lymphocyte sensitivity to amoebic antigen *in vitro*. That group which had passed cysts of *E. histolytica* at any time during the same period had, as a group, no significant lymphocyte sensitivity to amoebic antigen *in vitro*, although antibody levels were raised in both groups.

We would suggest a dual function and nature for cell-mediated responses in amoebic infection. Our results confirm that amoebiasis exists in an exposed, predominantly rural population largely as a subclinical,

symptomless infection. The constant, raised antibody levels in the population and low level lymphocyte sensitivity in consistently negative individuals support the hypothesis that this status is maintained, at least in part, by an effective immune response. Symptomless carriers show raised antibody levels but negative lymphocyte transformation, suggesting that increasingly successful *E. histolytica* invasion with micro-ulceration at the subclinical level may be associated with an increasing degree of immunodepression, but operating on the afferent pathway rather than inhibiting or destroying effector cells as no central sensitization can be detected. The interaction between amoebae and leucocytes has been studied (GRIFFIN, 1972; CHÉVEZ & SEGURA, 1974); amoebae are generally not killed by cells from naturally immune animals, from non-immune susceptible animals or from partially immune patients. After cure cells may be cytotoxic to amoebae (GUERRO *et al.*, 1975). This is clearly not a static situation, but will be represented as a dynamic spectrum from responsive, currently-immune individuals, through intermediates of decreasing sensitivity and increasing susceptibility to non-responsive, possibly immunodepressed, currently-vulnerable individuals.

Clinical amoebiasis develops when, as the result of the combination in many permutations of several factors—which we have discussed elsewhere (BRAY & HARRIS, 1977)—an increasing degree of immunodepression is induced. This may then be exploited and amplified by the amoebae so that they successfully invade and colonize to an extent causing clinical disease. Some degree of immunodepression will continue.

After invasion has occurred and, for one reason or another, amoebae commence to die, antigen becomes available to central immunocompetent cells, and sensitization of these cells is detected as specific lymphocyte transformation. After this point amoebae must operate their immunodepression on both afferent and efferent pathways to survive. This results in the characteristic lack of cellular response associated with the gut or liver lesion despite central sensitization.

Following clinical disease and cure or partial cure, the patient is left with a central population of sensitized lymphocytes. Locally, the situation is relatively unchanged, with amoebae able to exert an afferent immunodepression and thereby avoid alerting the immune system which would give a local response. A second invasion will again be preceded by circumstances resulting in a degree of local and central immunodepression and, although a brisker secondary response may occur, the timing of this will preclude any value in preventing a second attack of disease.

Cell-mediated immunity in amoebiasis exists, therefore, firstly as part of the normal, subclinical struggle between parasite invasion and host response, and secondly in the form of typical cellular responses to antigen following clinical invasion of the parasite. This hypothesis is intended to indicate a general trend in subclinical and clinical amoebiasis, and is not intended as a dogmatic statement of events.

The hypothesis then includes the postulate that consistently negative individuals in a population at constant risk would show an activated cell response to amoebae. It is unrealistic to expect a clear-cut result in these circumstances from a study of a few individuals and, as we would expect, we were able to detect only very few subjects who were consistently negative for infection. The figures adduced for cellular sensitivity were not inconsistent with our earlier hypothesis but clearly this must still remain speculative until further data, not necessarily from epidemiological studies, are collected.

We had hoped to follow the cellular and humoral antibody responses of amoebiasis patients, relating changes in immune parameters to clinical progress. However, clinical amoebiasis is rare in The Gambia, and the number of patients examined was really too small to draw any but speculative conclusions.

Over-all, in patients suffering from intestinal disease, little or no cellular sensitivity could be detected by our microculture method though individuals showed small rises in sensitivity. A low grade IgM response was seen by immunofluorescence with titres ranging from negative to 1:20, together with a moderately high IgG response—the titres in this case ranging from 1:20 to greater than 1:500. Cellular sensitivity remained negative with time in this group, and at four weeks after cure the IgM had disappeared, although the IgG remained elevated at this time.

In patients with liver abscess, a positive lymphocyte transformation response was seen on admission, which disappeared over approximately four weeks following successful treatment. Immunofluorescent IgM levels were high on admission, with titres from 1:20 to 1:320, but became negative following successful cure; IgG levels were very high on admission, with titres from 1:160 to greater than 1:1000, and generally remained high over the study period.

On first analysis, these results appear in contrast to the previously reported data on cellular sensitivity in clinical hepatic amoebiasis (LANDA *et al.*, 1975) where negative cellular responses were recorded on admission but becoming positive following successful treatment. The difference probably lies in the clinical condition of the patient. Our subjects were generally in quite good clinical condition on admission, and may therefore have a more vigorous ongoing response to the invading organism, and a readily detectable, circulating sensitized cell population. Conversely, patients in the previously-reported studies have been critically ill on admission, and may therefore have a less vigorous response, and quite possibly a suppressed circulating lymphocyte population. We know from other studies that *in vitro* cellular sensitivity can be very markedly influenced by the clinical state of the subject, and even a quite trivial infection may substantially alter the response of the subject's cells both to antigen and to PHA. Another unknown factor is the levels of metronidazole present in the blood at the time of the tests as metronidazole is known to suppress cellular sensitivity to mitogens.

The comparatively rapid disappearance of the detectable antigen-sensitive population of cells was also unusual, particularly in relation to the reported persistence of delayed skin sensitivity and MIF production following cure. The reasons for this were again not clear but we know that, although the triggering event of antigen binding may be the same, there are probably substantial differences in the mechanics and dynamics of lymphocyte transformation, lymphokine production and *in vitro* delayed hypersensitivity, and it is probably not realistic to try and draw close parallels between these criteria at this stage.

In The Gambia this host-parasite relationship probably exists in the form of a continuous trickle-type challenge in response to which a partial, non-sterile immunity develops, at least in terms of clinical disease although not in terms of actual infection. Superimposed on this will be

an increase of transmission during the humid rainy period causing the peak of infection during the early rains, but then occurs the sharp drop-off in infection during the late rains when we would expect any increased transmission to be continuing. In view of the increased challenge that must be occurring at this time of year—whether due to increased activity of the parasites or to an increase in the number of new infections—we would suggest that the sharp drop in infection during the latter part of the rains is due to a self-cure type of phenomenon. For the present the term is used in a purely descriptive sense and it is too early to speculate as to a possible mechanism, or even to suggest a parallel with other parasite systems where self-cure is known to occur. The very sharp nature of this peak, and particularly of the drop in August–September, argue against the possibility that this represents just a change in parasite demonstrability or some other epidemiological phenomenon.

A self-cure type of reaction will not give any lasting protection and a second peak of infection was seen reaching a maximum two to three months after the last rain. The drop in January and February we believe is due to the sharp onset of the dry season causing a sharp drop in transmission.

Within our villages, there was some evidence for compound clustering, which could suggest that the initial infection came from the food handler. This is not necessarily so, however. In these villages the distance between house and defaecation site was directly related to age with two to four-year-old children defaecating immediately around or actually in the compound. Peridwelling faeces from infected toddlers may be the principal vehicle of transmission. This type of distribution would ensure widespread dispersal and could lead to the almost universal incidence of asymptomatic infection recorded; it could, by infecting the mother (and pre-adolescent "aunties"), lead to the higher infection prevalences in females at any one point in time.

The lack of success in our attempts to elucidate the transmission pathway was remarkable. Amongst the study population, almost everybody over the age of five years passed cysts of *E. histolytica* at some time over the year, so dissemination of cysts must be widespread. The culture methods we have used are sensitive, detecting probably a minimum of five to 20 cysts, and we had expected to concentrate this number of cysts from the comparatively large quantities of material collected. It may be of course that we were either looking in the wrong places, in which case the classical paths of transmission were definitely not being followed in The Gambia, or that small numbers of cysts were distributed by each of many different routes. If this were so, then by looking at any one route we may not have been able to collect adequate numbers of cysts to be detected by our methods. This result does serve to emphasize the need to study transmission routes in particular situations, rather than to assume that classical pathways are operating.

In the bush, transmission person-to-person or self-reinfection, as a single event must be relatively infrequent as the population density is low and the environment is inimical to cysts for much of the year. Rapid faecal transmission, occurring here only during the rains, appears to be an essential process for the development of maximum pathogenic potential in man (POWELL, MADDISON & ELSDON-DEW, 1966). The highly seasonal mode of transmission in The Gambia may thus prevent the development of the full pathogenic potential of local strains of parasite. Those very small areas of the country where urbanization may encourage more continuous transmission have only been in existence for a comparatively short period of time, and we may see the emergence of increasingly pathogenic amoebae in these areas with time.

There was no evidence to suggest that local strains were inherently non-pathogenic. 18 local strains of parasite were isolated in Robinson's medium from stools collected on the survey. None of these grew at room temperature, and all had cysts of above 10 mc in diameter. Occasional *E. hartmanni* and uninucleate cysts of presumably *E. polecki* were found. Further studies on the experimental pathogenicity of local strains and, for example, on DNA buoyant density and iso-enzyme analysis would be very valuable.

References

André, L. J. (1971). *Amibiase*, Monographie SPECIA, Paris.

Bran, J. L. (1974). Epidemiology. In: Padilla y Padilla, C. A. and Padilla, G. (Editors). *Amebiasis in Man: Epidemiology, Therapeutic, Clinical Correlations and Prophylaxis*. Illinois: Charles C. Thomas, pp. 351–360.

Brandt, H. & Perez-Tamayo, R. (1970). Pathology of human amoebiasis. *Human Pathology*, **1**, 351–360.

Bray, R. S. & Harris, W. G. (1977). *Entamoeba histolytica*. Unitarians and pluralists; Prometheans and commensalists. *Protozoology*, **3**, 193–196.

Chévez, A. & Segura, M. (1974). Interacciòn entre los trofozoítos de *E. histolytica* y los leucocitos de varias; especies animales. *Archivos de Investigacion Medica*, **5**, Suppl. 2, 373–382.

Dobell, C. & Laidlaw, P. P. (1926). On the cultivation of *Entamoeba histolytica* and some other entozoic amoebae. *Parasitology*, **18**, 283–318.

Elsdon-Dew, R. (1968). The epidemiology of amoebiasis. *Advances in Parasitology*, **6**, 1–62.

Griffin, J. L. (1972). Human amoebic dysentery. Electron microscopy of *Entamoeba histolytica* contacting, ingesting and digesting inflammatory cells. *American Journal of Tropical Medicine and Hygiene*, **21**, 895–906.

Guerrero, M., Landa, L. and Y. Rios, D. (1975). Interaction between trophozoites of *E. histolytica* and lymphocytes with invasive amoebiasis. *Resumenes de la Conferencia Internacional sobre Amibiasis (Ciudad de Mexico)*, p. 88.

Harris, W. G. & Bray, R. S. (1976). Cellular sensitivity in amoebiasis—Preliminary results of lymphocyte transformation to specific antigen and PHA in carrier and disease states. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **70**, 340–343.

Jenkinson, S. G. & Hargrove, M. G. (1975). Recurrent amebic abscess of the liver. *Journal of the American Medical Association*, **232**, 277–278.

Kagan, I. G. (1973). The immunology of amebiasis. *Archivos de Investigacion Medica*, **4**, Suppl. I. s169–s176.

Kagan, I. G. (1974). Pathogenicity of *E. histolytica*. *Archivos de Investigacion Medica*, **5**, Suppl. 2, 457–464.

Kane, G. J., Matossian, R. & Batty, I. (1971). Fluorochrome-labelled anti-immunoglobulin fractions used with stabilized antigen preparations for the assessment of parasitic diseases. *Annals of the New York Academy of Science*, **177**, 134–145.

Knight, R. (1975). Surveys for amoebiasis. Interpretation of data and their implications. *Annals of Tropical Medicine and Parasitology*, **69**, 35–48.

Knight, R., Schultz, M. G., Hoskins, D. W. & Marsden, P. D. (1973). Progress report. Intestinal parasites. *Gut*, **14**, 145–168.

Krupp, J. (1970). Antibody response in intestinal and extra-intestinal amebiasis. *American Journal of Tropical Medicine and Hygiene*, **19,** 57–62.

Landa, L., Guerrero, M. & Capin, R. (1975). Studies on cellular immunity in invasive amebiasis. *Resumenes de la Conferencia Internacional sobre Amibiasis* (*Ciudad de Mexico*), p. 104.

Maddison, S. E., Kagan, I. G. & Norman, L. (1968). Reactivity of human immunoglobulins in amebiasis. *Journal of Immunology*, **100,** 217–226.

Maegraith, B. G. & Harinasuta, C. (1954a). Experimental amoebic infection of the liver of guinea pigs. I. Infection via the mesenteric vein and via the portal vein. *Annals of Tropical Medicine and Parasitology*, **48,** 421–433.

Maegraith, B. G. & Harinasuta, C. (1954b). Experimental amoebic infection of the liver of guinea pigs. II. Abscess formation in animals with persistent intestinal lesions. *Annals of Tropical Medicine and Parasitology*, **48,** 434–441.

Ortiz-Ortiz, L., Zamacona-Ravelo, G. & Capin, N. R. (1974). Hipersensibilidad celular en amibiasis. III. Efecto *in vitro* de la concanavilina a y de antigeno amibiasis sobre leucocitos periféricos de pacientes con absceso hepático amibiano. *Archivos de Investigacion Medica*, **5,** Suppl. 2, 481–486.

Powell, S. J., Maddison, S. E. & Elsdon-Dew, R. (1966). Rapid faecal transmission and invasive amoebiasis in Durban. *South African Medical Journal*, **40,** 646–649.

Robinson, G. L. (1968). The laboratory diagnosis of human parasitic amoebae. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **62,** 285–294.

Sapero, J. J. & Johnson, C. M. (1939). An evaluation of the role of the food handler in the transmission of amebiasis. *American Journal of Tropical Medicine*, **19,** 705, 255–264.

Savanat, T. (1968). Immunity in amoebiasis. *Proceedings of the Seminar on Filariasis and Immunology of Parasitic Infections. Laboratory Meeting*, 1968, pp. 22–38.

Savanat, T., Viriyanond, P. & Nimitmongkol, N. (1973). Blast transformation of lymphocytes in amebiasis. *American Journal of Tropical Medicine and Hygiene*, **22,** 705–710.

Schoenleber, A. W. (1940). The food handler as a transmitter of amebiasis. *American Journal of Tropical Medicine*, **20,** 99–106.

Stamm, W. P. (1970). Amoebic aphorisms. *Lancet*, **ii,** 1355–1356.

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