RAPID FAECAL TRANSMISSION AND INVASIVE AMOEBIASIS IN DURBAN

S. J. POWELL, M.D., M.R.C.P. (EDIN.), S. E. MADDISON, PH.D. (CAPE TOWN) AND R. ELSDON-DEW, M.D., F.R.S.S.A., *Amoebiasis Research Unit, Durban*

There are many factors to be considered in accounting for the contrasting manifestations of infection with *Entamoeba histolytica* in tropical and temperate zones. Though commoner in warm climates the parasite does occur in colder parts of the world. However, invasion of the tissues is rare in temperate zones in contrast to the picture in some warmer areas.

In Durban, where invasive amoebiasis is commoner among Africans than in either Indian or White populations, climate *per se* can be excluded as a factor accounting for the difference in prevalence. Severe manifestations are frequent among Africans but, when the diagnosis is indisputable, the disease takes the same form in all 3 races and, for that matter, anywhere else in the world. The unwarranted association of the parasite with vague syndromes initially gave the impression that the condition took different forms in different peoples and locations, but once it was appreciated that the amoeba can, and usually does, live in harmony with its host, the disease became a well-defined entity. Is the frequency of invasion in the African then due to factors in the parasite, in the host or in the environment?

Is a different species of amoeba responsible for the invasive type of infection? In the past, the morphological similarity of the commensal *Entamoeba hartmanni* to *E. histolytica* has confused the issue,[1] but when identification of *E. histolytica* has been certain there is no evidence of a species difference between invasive and non-invasive forms, although in the invasive case the amoeba becomes haematophagous. However, the possibility of strain differences or of acquired alteration in virulence remains, and is discussed later.

Does the difference lie in the host? The high incidence of invasive amoebiasis among Africans in Durban does not necessarily imply a racial difference as the environments of the 3 races differ. Furthermore there are other parts of the continent where Africans do not seem more susceptible than other races.

Dietary differences were at one time thought to play some part, possibly by sustaining a bowel flora favourable to invasion by amoebae.[2] Though differences in bowel flora remain of possible importance in determining invasiveness, we have not yet been able to relate the occurrence of the disease to any specific form of malnutrition in our patients. The disease, in fact, is common in otherwise healthy, young wage-earning African males. Nor have we been successful in converting human cyst-passers to the invasive state by variations in diet. Similar experiments in monkeys previously colonized with *E. histolytica* have also failed.[3]

Do other organisms play some part in initiating invasion? The possibility originally suggested by Westphal[4] that some infective agent might change a cyst-passing commensal condition to the invasive state led us to attempt such conversion in naturally infected baboons by the administration of fresh dysenteric faeces.[5] This failed, none of the 9 animals developing any symptomatology.

Do helminths play a role? In Durban helminth infections are exceedingly common in both Indians and Africans, although in an early study of predominantly dysenteric stool specimens the prevalence of protozoa other than *E. histolytica* was low.[6] Subsequent comparison of patients with dysentery and a control group showed a much lower incidence of other protozoa in the dysenteric specimens,

and it appears that the mere presence of dysentery greatly reduces the load of protozoa; the distribution of helminth ova is apparently not similarly affected and there was no positive evidence relating invasion by *E. histolytica* to their presence.[7]

The relationship between some environmental factor and invasive amoebiasis was suggested by the original observation,[8] subsequently confirmed, that by far the heaviest incidence was in the notorious slum, Cato Manor. Removal of the inhabitants to modern townships was followed by a notable fall in frequency of the disease. Although there has been no coincident change in dietary habits, the sanitation and water supplies for the African community have been much improved. Moreover, the initial study on Cato Manor patients indicated an association between the occurrence of cases and rainfall, suggesting contamination of drinking water with fresh sewage. Faecal contamination of water has been found in almost all adequately investigated outbreaks of amoebiasis in other parts of the world[9,10] and, apart from such outbreaks, in countries with advanced standards of hygiene, such as the USA, a high prevalence of amoebiasis is virtually limited to mental institutions where the habits of the patients facilitate the direct transmission of faecal material.[9]

Faust[11] claimed that the incidence of *Entamoeba coli* in a community could be used as an index of the rapidity of transmission of faecal material from one individual to another. Brooke *et al.*[12,13] subsequently introduced the more accurate 'combined amoebic prevalence rate' based on the accounting of individuals harbouring one or more of the common species of amoebae, all of which are transmitted as cysts in the same manner. Whereas these protozoa need relatively rapid passage from host to host in order to spread in a community, the ova of the commoner helminths become viable only after a period of weeks. Contaminated soil remains infective for a considerable time so that such helminth infections originate from long-polluted soil rather than from freshly contaminated water or food.

The present study was undertaken to provide information on the relative prevalence of intestinal parasites in non-dysenteric African and Indian patients in order to determine whether such a difference in the pattern of faecal transmission might account for the difference in incidence of invasive amoebiasis in the 2 communities.

MATERIALS AND METHODS

Three successive stool specimens were examined from 165 African, 144 Indian and a further 30 African patients in whom cysts of *E. histolytica* had been found on previous examinations. All were adult patients at King Edward VIII Hospital, Durban, and none had dysentery or clinical evidence of amoebiasis.

Specimens were examined by the direct and zinc sulphate flotation technique and egg counts were done by the method of Beaver[14] on 1 - 2 mg. faecal smears.

RESULTS

As shown in Table I the Indian and African groups have a similar high prevalence of soil-transmitted helminths although trichuris and ascaris are commonest among African cyst-passers, a finding comparable to that in amoebic dysentery.[7] Despite the high prevalence of helminth infection in Indians, the incidence of all protozoa is much lower in this group than in the Africans among whom the cyst-passers show the highest frequency. A combined amoebic index has been included in Table I. In determining this index, those persons are counted who are infested with 1 or more of the 5 common species of amoebae (*E. histolytica, E. hartmanni, E. coli, Endolimax nana* and *Iodamoeba butschlii*). In the group of *E. histolytica* cyst-passers the index has been determined from the presence of the other 4 species of amoebae. The index shows an extremely high prevalence of other protozoal infections in the *E. histolytica* cyst-passers and a much higher level of infection in Africans than in Indians.

TABLE I. PERCENTAGE PREVALENCE OF INTESTINAL PARASITES

	Africans	*Indians*	*Cyst-passers*
No. of cases examined:	165	144	30
Metazoa			
Trichuris—total	59	63	74
Ascaris—total	38	30	50
Hookworm—total	20	17	17
Strongyloides	3	4	3
Hymenolepis	1	0	0
Taenia spp.	10	1	13
Enterobius	0	2	0
S. mansoni	1	2	0
S. haematobium	0	0	0
Protozoa			
E. histolytica	14	1	100
E. coli	38	8	70
E. hartmanni	13	2	37
E. nana	12	4	40
I. butschlii	10	0	27
C. mesnili	7	1	20
G. lamblia	5	3	3
T. hominis	5	0	0
Isospora	3	1	3
Combined amoebic index	52	10	84

The incidence and degree of trichuris, ascaris and hookworm infection in the 3 groups is shown in Table II. Despite their high prevalence it is exceptional for these infections to be heavy, a finding previously reported in our patients with dysentery.[7]

TABLE II. DEGREE AND INCIDENCE OF TRICHURIS, ASCARIS AND HOOKWORM INFECTIONS (IN PERCENT)

	Africans	*Indians*	*Cyst-passers*
No. of cases examined:	165	144	30
Trichuris			
Light	55	58	70
Moderate	4	4	2
Heavy	0	1	2
Total	59	63	74
Ascaris			
Light	34	24	40
Moderate	2	4	7
Heavy	2	2	3
Total	38	30	50
Hookworm			
Light	19	15	17
Moderate	0	2	0
Heavy	1	0	0
Total	20	17	17

DISCUSSION

Parasites with a similar pattern of transmission will occur together more frequently than when there are differences in the mechanism of passage. The statistical approach is indicated in the appendix and shows the close association between ascaris and trichuris, both of which species undergo a period of development in the soil and are ingested. There

is also a close association between *E. histolytica* and 'other protozoa', indicating the similarity of the method of transmission. On the other hand, the low correlations shown between *E. histolytica* and the helminth infections suggest a difference which may well be due to the interval between extrusion from one host and ingestion by the next, a long period favouring, and a short period being unsuitable for, the helminths, whereas the contrary probably holds for the protozoa.

As helminth infections are frequent and similar in degree among Indians and Africans in Durban, transmission is equally successful in both groups and indicates similar exposure to long-polluted soil. On the other hand the high incidence of protozoal infection in the African, as shown by the rates for *E. coli* and the combined amoebic index, is an indication that they are more exposed to fresh faeces than is the Indian community. Such exposure to rapid faecal transmission is also evidenced by the high prevalence of other diarrhoeal and dysenteric disorders in the African. As far back as 1913, Walker[15] pointed out the similarity in epidemiology between amoebiasis and typhoid fever, and in our hospital Africans show a higher incidence of the latter than do Indians. The prevalence of typhoid fever, like that of invasive amoebiasis, appears to be falling concurrently with the provision of better housing and sanitation.

As at one time it was believed that the incidence of *E. histolytica* was similar in the 3 racial communities in Durban,[16] additional factors, such as feeding habits peculiar to the local African, were postulated to account for the invasiveness of the amoebae among them. However, more recent studies[17] (confirmed by the present study) showed that the incidence of *E. histolytica* is, by comparison, low in the local Indians—an observation confirmed by the low incidence of antibodies to amoebae found in both Indian and White communities in Durban.[17]

Though the rapid transfer of fresh faeces explains the higher incidence of *E. histolytica* in the African, it does not *per se* indicate the mechanism underlying the high prevalence of invasive amoebiasis among them. Is this due to dosage, to a gain in virulence or to some other factor?

Estimation of the effect of dosage in an organism which multiplies after ingestion is difficult. Nevertheless, in experimental infections the size of the initial dose of amoebae is of importance in determining whether or not successful colonization will take place, and heavy doses are more likely to overwhelm the defences in humans.

Does the amoeba gain in virulence by rapid passage? There is no laboratory animal in which it has been possible adequately to reproduce the human pattern of both commensal and invasive amoebiasis. Primates harbour the amoeba in the commensal phase and disease does not ensue. However, in rodents, artificially infected via the caecum, Neal[18] has shown that intestinal passage revives the virulence of attenuated strains of *E. histolytica,* and it has also been demonstrated that serial liver passage in hamsters increases virulence.[19] In our own studies of experimental liver abscess in hamsters we have suggested that amoebae obtained from our patients with amoebic liver abscess have attained their maximum virulence.[20] Although adequate experimental proof is still lacking, a gain in virulence resulting from rapid transmission from person to person may well provide the explanation for the frequency of invasion in the African.

The precise mechanism accounting for this increase in virulence is unknown but this could be due to the passage of some labile 'trigger' factor. The effect of 'viral' infection of *Corynebacterium diphtheriae* on its virulence provides a possible analogy. The recent discovery by Tomasz[21] of 'activator' substances inducing genetic transformation in pneumococci and other organisms raises yet another possibility. However, until some reliable parameter for virulence in *E. histolytica* is established, such considerations must remain speculative.

Although the details of the mechanism responsible for variations in the pathogenicity of *E. histolytica* have not been established, the present report indicates the importance of rapid faecal transfer in the epidemiology of amoebiasis and, in doing so, points the way to control of the disease.

APPENDIX

If different parasites have a common factor in transmission, their simultaneous appearance in a host will be more frequent than if no common factor existed and their occurrence was randomly distributed. This can be illustrated from the above material in 4-fold tables (Table III A, B, C, and D).

TABLE III. FOURFOLD ASSOCIATIONS

A		*Ascaris* +	*Ascaris* −	
Trichuris	+	·2714 **·2112**	·3810 **·4412**	·6524
	−	·0524 **·1126**	·2952 **·2350**	·3746
		·3238	·6762	

B		*E. coli* +	*E. coli* −	
I. butschlii	+	·1000 **·0459**	·0095 **·0636**	·1095
	−	·3190 **·3731**	·5714 **·5714**	·8905
		·4190	·5801	

C		*Ascaris* +	*Ascaris* −	
Hook/Strong.	+	·2238 **·1947**	·0524 **·0815**	·2762
	−	·4810 **·5101**	·2429 **·2136**	·7238
		·7048	·2952	

D		*E. coli* +	*E. coli* −	
Hook/Strong.	+	·1286 **·1119**	·1476 **·1552**	·2762
	−	·2905 **·3032**	·4333 **·3376**	·7238
		·4190	·5810	

Bold figures give random distribution.

It will be seen from Table IIIA that ascaris and trichuris, both needing a period of soil development, show a much higher association (0·2714) than if they were randomly distributed (0·2112). Similarly *E. coli* and *I. butschlii* are twice as commonly associated than predicted by random distribution.

These associations can be measured by Pearson's cosine method of determining the coefficient of correlation r and the statistical significance of the observation by X^2 with a corresponding probability P for one degree of freedom. The coefficient of correlation r varies between zero for no correlation and 1 for complete association.

These are set out in Table IV, for some combinations and permutations on the data from African subjects. It will be seen that *E. histolytica* is closely associated with 'other protozoa' and with a high degree of confidence. It is thus legitimate to use the incidence of 'other protozoa' as an index of the likelihood of infection with *E. histolytica.* The 2 groups have a

common factor which is almost certainly the rapidity with which faeces from one individual reach the intestine of another.

TABLE IV. ASSOCIATION BETWEEN DIFFERENT PARASITES

			r	X^2	P
Ascaris	v.	Trichuris	0·90	15·3	<0·001
Ascaris	v.	Hookworm and/or strongyloides	0·30	4·3	0·02–0·05
E. coli	v.	Hookworm and/or strongyloides	0·07	0·7	0·70–0·80
E. coli	v.	*I. butschlii*	0·83	25·9	<0·001
E. histolytica	v.	*E. coli*	0·41	7·59	0·001–0·01
E. histolytica	v.	Ascaris and/or trichuris	0·32	2·42	0·10–0·20
E. histolytica	v.	Hookworm and/or strongyloides	0·07	0·19	0·50–0·70
E. histolytica	v.	All other protozoa	0·77	11·9	<0·001

SUMMARY

Comparison of the prevalence of intestinal parasites in non-dysenteric African and Indian patients at King Edward VIII Hospital, Durban, shows a high incidence of soil-transmitted helminths in both races, although the degree of infection is light in the vast majority. In contrast, the incidence of intestinal protozoa is much higher among Africans, being highest in those harbouring cysts of *E. histolytica*. It is suggested that these differences are due to different patterns of transmission, and statistical analysis of these findings supports this view. The rapid transfer of fresh faeces can explain the higher incidence of *E. histolytica* among Africans and a gain in virulence due to repeated passage may account for the frequency of invasive amoebiasis. The underlying mechanism accounting for increase in virulence is briefly discussed.

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