Library (2)

Attachment 1.

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Date 24/9/89

Principal Investigator Dr. Andrew Hall

Application No. 89-007

Title of Study Establishment of techniques to identify pathogenic strains of Entamoeba histolytical...in Bangladesh

Trainee Investigator (if any) _____

Supporting Agency (if Non-ICDDR,B) _____

Project status:
( ) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

Circle the appropriate answer to each of the following (If Not Applicable write NA).

Source of Population:
(a) Ill subjects — (Yes) No
(b) Non-ill subjects — (Yes) No
(c) Minors or persons under guardianship — Yes No

Does the study involve:
(a) Physical risks to the subjects — Yes (No)
(b) Social Risks — Yes (No)
(c) Psychological risks to subjects — Yes (No)
(d) Discomfort to subjects — Yes (No)
(e) Invasion of privacy — Yes (No)
(f) Disclosure of information damaging to subject or others — Yes (No)

Does the study involve:
(a) Use of records, (hospital, medical, death, birth or other) — (Yes) No
(b) Use of fetal tissue or abortus — Yes (No)
(c) Use of organs or body fluids — Yes (No)

Are subjects clearly informed about:
(a) Nature and purposes of study — Yes (No)
(b) Procedures to be followed including alternatives used — Yes (No)
(c) Physical risks — Yes (No)
(d) Sensitive questions — Yes (No)
(e) Benefits to be derived — Yes (No)
(f) Right to refuse to participate or to withdraw from study — Yes (No)
(g) Confidential handling of data — Yes (No)
(h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure — Yes (No)

N/A

5. Will signed consent form be required:
   (a) From subjects — Yes (No)
   (b) From parent or guardian (if subjects are minors) — Yes (No)
6. Will precautions be taken to protect anonymity of subjects — (Yes) No
7. Check documents being submitted herewith to Committee:
   ___ Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
   ___ Protocol (Required)
   ___ Abstract-Summary (Required)
   ___ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
   ___ Informed consent form for subjects
   ___ Informed consent form for parent or guardian
   ___ Procedure for maintaining confidentiality
   ___ Questionnaire or interview schedule *

* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:
1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will be presented to the Cttee. for review.

(PTO)

ee to obtain approval of the Ethical Review Committee for any changes ing the rights and welfare of subjects before making such change.

Principal Investigator

DEC 04 1989

Trainee

89-007
25/7/89

RESEARCH PROPOSAL

INVESTIGATORS	Andrew Hall Rashidul Haque John Ackers (London School of Hygiene and Tropical Medicine)
COORDINATOR	Saul Tzipori S. Tzipori
TITLE	Establishment of techniques to identify pathogenic strains of Entamoeba histolytica for use in clinical and epidemiological studies in Bangladesh.
STARTING DATE	When the money is available
DATE OF COMPLETION	Three years after start
TOTAL BUDGET	US$ 116,872
FUNDING SOURCE	

AIMS

General aim

To establish the significance of Entamoeba histolytica as a cause of dysentery in Bangladesh.

Specific aim

To establish techniques of isoenzyme electrophoresis to distinguish between pathogenic and non-pathogenic strains of E.histolytica isolated in Bangladesh.
To compare the results of isoenzyme electrophoresis with an immunodiagnostic test using a monoclonal antibody which is claimed to distinguish between pathogenic and non-pathogenic strains.
To use these techniques in epidemiological studies of E.histolytica as a cause of dysentery in Bangladesh.
To establish the carriage rate of pathogenic and non-pathogenic zymodemes in Bangladesh.
To establish serological tests to diagnose amoebiasis and to study the processes of disease.
To use these techniques to assist in clinical studies of dysentery at the ICDDR,B.

Significance

This research will contribute to knowledge about the role of E.histolytica as a cause of disease in Bangladesh.

A-033781

ETHICAL IMPLICATIONS

There are no aspects of this research which are harmful to humans.

BACKGROUND

Entamoeba histolytica is the cause of amoebic dysentery, a disease caused when organisms invade the gut wall. Serious complications may arise when amoebae are washed in the blood stream to internal sites such as the liver, where they may cause potentially lethal abscesses.

There is growing evidence from studies of Entamoeba histolytica throughout the world that there are at least 20 different strains of the species. These strains are indistinguishable on the basis of their microscopical appearance but they can be distinguished by the electrophoresis of enzymes extracted from lysed organisms. The strains identified by this technique are called "zymodemes", populations of organisms differing from other populations of the same species by the electrophoretic mobility of different molecular forms of the same enzymes, called "isoenzymes". Four enzymes are used to distinguish between species of amoebae and between strains of E.histolytica: hexokinase, malate enzyme, glucose phosphate isomerase and phosphoglucomutase (Sargeaunt, 1987a,b).

Several zymodemes of Entamoeba histolytica are strongly associated with the ability to cause disease: these strains can invade tissues, consume erythrocytes and produce abscesses. In contrast, the remaining zymodemes are not associated with disease and appear to live as non-invasive commensals of the intestinal lumen. There is a recent report of a non-pathogenic strain which changed in vitro to become pathogenic (Mirelman, 1987), but whether this can occur in vivo is unknown and requires investigation. Man is the only know host of E.histolytica.

The current state of knowledge and opinion about E.histolytica in Bangladesh can be introduced from two different perspectives, one parasitological and the other medical, and both reveal fundamental and important deficiencies in knowledge.

As an intestinal parasitic infection E.histolytica appears to be fairly common in Bangladesh, but there are few recent surveys which give data which is parasitologically credible, so two sources of information from research within the ICDDR,B during 1988 will be cited. First, the prevalence of E.histolytica among 1,820 slum-dwellers of all ages in Dhaka was found to be 7.7% (Hall, unpublished observations). Secondly, cysts or trophozoites of E.histolytica were found in 352 (1.8%) of 19,528 faecal samples examined in the Clinical Pathology Laboratory of the ICDDR,B during 1988. Of the 289 stools in which trophozoites were seen, 75 contained trophozoites with ingested red blood corpuscles indicating that they were pathogenic strains.

There are no reports of the zymodemes of Entamoeba histolytica present in Bangladesh and so there is no information about the prevalence of pathogenic strains. The only evidence is indirect. A study of sera collected in Matlab, Bangladesh concluded that 80% of 200 healthy villagers had antibodies to Entamoeba histolytica by the age of 14 years (Hossain et al. 1983). These figures are dubiously high when compared with figures from a serological survey of 20,000 people conducted in Mexico, a country in which amoebic dysentery is commonly diagnosed: antibodies to E.histolytica were found in only 6% of the Mexican sample (cited by Sepulveda & Martinez-Palomo, 1985).

The distribution of pathogenic zymodemes of E. histolytica has been shown to vary from country to country and reports from India indicate asymptomatic cyst passers are common in the sub-continent (Nanda et al., 1984). Nevertheless amoebiasis — as opposed to asymptomatic infections — does occur in Bangladesh and a series of 22 post mortems conducted on people who were suspected to have died due to amoebic dysentery at the ICDDR,B found characteristic lesions of the intestinal wall, but no abscesses on internal organs (Wanke et al., 1988). A series of 140 autopsies also conducted at the ICDDR,B found that amoebic colitis was an underlying cause of death in 13% of cases (Butler et al., 1987). Although E.histolytica may be diagnosed less commonly in patients at an urban diarrhoeal diseases hospital (1.8%) than in people living in an urban slum (7.7%), it appears to be strongly associated with deaths in hospital.

It appears to be widely perceived by physicians in rural Bangladesh that amoebiasis is common. A survey of allopathic drugs prescribed for 198 rural children with diarrhoea showed that metronidazole was the most commonly prescribed drug which could be identified from tablets still in the household (Ronsmans, unpublished data). Furthermore, of 150 physicians asked what drug they would give to a 2 year old child with dysentery, metronidazole was prescribed by 71. Metronidazole is a toxic drug which is effective against many species of anaerobic bacteria and against intestinal protozoa, but has no significant effect on species of Shigella

The apparent preference of physicians in Bangladesh to treat dysentery with metronidazole should also be seen in the light of the fact that shigellosis is undoubtedly common in Bangladesh: in 1988 Shigella was isolated from 16% of stool samples tested by the ICDDR,B in Dhaka, from 17% in Matlab and from 45% in Teknaf (ICDDR,B, 1989). Furthermore in a microbiological and parasitological survey of 101 people who claimed to have dysentery in northern Bangladesh, nobody was found to be infected with E. histolytica while 50% were infected with Shigella (Ronsmans et al., 1988). This raises the first point to be addressed by this research: that there is a need for information about the relative prevalence of amoebic and bacillary dysentery in Bangladesh so that the probability of prescribing an effective drug on the basis of symptoms alone is as high as possible.

But simply diagnosing E.histolytica is not sufficient, either in cases of dysentery or in asymptomatic infections, because it will be necessary to distinguish between pathogenic and non-pathogenic zymodemes. In cases of dysentery, if a pathogenic zymodeme is detected, a diagnosis of amoebic dysentery can be made; if a non-pathogenic zymodeme is detected in a patient with dysentery - then some other cause must be sought. This may occur because if the prevalence of E.histolytica is taken to be 10% then it is likely that 10% of people infected with Shigella will be infected with both organisms, as will 10% of people who have non-Shigella dysentery. So distinguishing between zymodemes will be an important part of estimating the role of E.histolytica as a cause of dysentery in Bangladesh.

It will also be important to estimate the carriage rate of pathogenic zymodemes among asymptomatic people. Although it can be argued that all infections with E.histolytica should be treated, it is perhaps better to take such a decision on the basis of relative risks: the risk of amoebiasis if pathogenic strains of E.histolytica are uncommon, the risk of reinfection after treatment, and the risk of toxic side effects of metronidazole. For example, it is now practice in the UK not to treat infections with E. histolytica in homosexuals because infections with zymodemes associated with invasiveness are rare and the chance of reinfection among homosexuals is high (Goldmeier et al., 1986).

In order to provide information about the relative importance of bacillary and amoebic dysentery and about the prevalence of pathogenic strains of E.histolytica, it will be necessary to undertake epidemiological surveys in several settings. Ideally such surveys should be conducted in both urban and rural areas, and in both hospitals and the community. People of all ages should be studied. The methods to undertake such research are well established and are available within the ICDDR,B.

Entamoeba histolytica and other amoebic infections of man can be isolated and easily grown in vitro by inoculating fresh faeces directly into a simple culture medium (Robinson, 1968). These primary cultures are grown with Escherichia coli and can usually be used within a few days for isoenzyme electrophoresis both to confirm the diagnosis of a species of amoeba made by microscopy, and to distinguish between zymodemes. Entamoeba histolytica has been established in Robinson's medium at the ICDDR,B, while isoenzyme electrophoresis is already being done for 34 enzymes of Giardia intestinalis, including the 4 enzymes which distinguish between strains of E.histolytica.

In addition, if the organisms are established in axenic culture, it will be possible to make available to the Centre's physicians serological tests for amoebiasis. At the Amoebiasis Unit of the Hospital for Tropical Diseases in London (the U.K. national reference centre for amoebiasis), serum is first screened by indirect fluorescence antibody tests and if

antibodies are detected at a titre of greater than or equal to 1:80 a cellulose acetate precipitin test is done (Moody, Personal Communication). Such tests can be established easily once a source of antigen is available and a local screening titre can be established.

One of the investigators (JA) has been recently involved in the production of a monoclonal antibody which appears to bind exclusively to isolates of E. histolytica with fast hexokinase bands — a marker of pathogenicity (Strachan et al. 1988). This monoclonal antibody will be compared with isoenzyme electrophoresis to detect pathogenic and non-pathogenic strains of E.histolytica.

PLAN OF RESEARCH

Parasitological techniques

These are well established and will only be outlined here. To isolate and culture E.histolytica, fresh stools are inoculated into Robinson's medium (Robinson, 1968) and incubated for between 48 and 72 hours. Trophozoites are harvested when there are about 10^5 organisms and then lysed by freezing and thawing in the presence of enzyme stabilisers (Sargeaunt & Williams, 1978). After removing cell debris by centrifugation, drops of supernatant are frozen in liquid nitrogen to prepare beads. These beads are then unfrozen when required and applied to plates in cellulose acetate electrophoresis or are soaked into cotton threads for starch gel electrophoresis. To distinguish between zymodemes, isoenzyme electrophoresis is performed for the enzymes glucose phosphate isomerase (EC 5.3.1.9), phosphoglucomutase (EC 2.7.5.1), hexokinase (EC 2.7.1.1) and L-malate: NADP+ oxidoreductase (oxaloacetate decarboxylating). Both cellulose acetate and starch gel electrophoresis will be undertaken, but every effort will be made to use only cellulose acetate electrophoresis because of its simplicity and convenience. If three beds are available then only one large bead would be required to screen one isolate for all enzymes.

Controls of pathogenic and non-pathogenic zymodemes will be obtained from the London School of Hygiene and Tropical Medicine. The isoenzyme patterns of zymodemes of E.histolytica isolated in epidemiological studies will be classified according to the most recent zymodeme pattern available (Sargeaunt et al., 1978; Sargeaunt, 1987b). Isolated organisms will be stained if necessary to confirm the species of amoeba, and cloned if there is evidence of more than one zymodeme in an isolate. Stocks of different zymodemes will be stored in liquid nitrogen.

Organisms will be established in axenic culture in Diamond's medium (Diamond et al. 1978) and used to provide antigens to develop immunological tests for infection including a screening test by indirect fluorescence and a cellulose acetate precipitin test (Stamm & Phillips, 1977).

Epidemiological techniques

As stated above, the ideal research to provide answers to the aims would be to estimate the prevalence of pathogenic and non-pathogenic strains of E.histolytica in people of all ages living in both rural and urban communities, and the prevalence of amoebic infection and amoebiasis among patients who come for treatment at both rural and urban hospitals. However it is recognised that this would be a considerable and expensive undertaking. For this reason it is planned to begin work with a study based in a hospital (Phase 1), a situation which should result in some selection for amoebiasis and would establish the role of E.histolytica as a cause of dysentery.

Phase 1: an urban hospital. All faecal specimens collected as part of the Surveillance System being conducted at the Dhaka Treatment Centre of the ICDDR,B which are submitted to the Clinical Pathology Laboratory for microscopical examination will be inoculated into Robinson's medium according to the methods described above. As a part of the surveillance system information is routinely recorded about the clinical characteristics of all patients being studied and a stool sample from each patient is cultured for Salmonella spp, Shigella spp and for Vibrio cholerae. This information will provide a ratio of infections with Shigella to E.histolytica and of the clinical characteristics of patients infected with E.histolytica.

In 1988 a total of 2408 samples were examined in the Clinical Pathology Laboratory for the Surveillance System: if the incidence of E.histolytica is taken to be 1.8% then only about 40 would have contained E.histolytica. Because this is a small sample, in addition to the specimens from the Surveillance System, all stools seen to contain trophozoites or cysts of amoebae will also be inoculated into Robinson's medium by the staff of the Clinical Pathology Laboratory. This will serve two purposes: to determine the proportion of diagnosed infections with E.histolytica which are of pathogenic zymodemes and secondly, to confirm the diagnosis of E.histolytica made by microscopy. This sampling procedure would result in between 50 and 60 bottles of Robinson's medium a week being prepared for culture, of which between 5 and 10 are likely to contain E.histolytica. As the rate limiting step in this research is isoenzyme electrophoresis, this number of samples is considered to be adequate at the outset: if more samples can be processed then the collection of samples will be expanded as outlined below. The culture and isoenzyme electrophoresis of amoebae found will be offered as a diagnostic test.

Phase 2: a rural hospital. Faecal samples will be collected from the Centre's hospital at Matlab Field Station. A microscopical examination of faeces is not done routinely in Matlab but for the last 3 years more than 5000 faecal samples have been cultured each year. A sampling procedure would be worked out by discussion with the staff of the Field Station and the practicalities of sending to Dhaka bottles of Robinson's medium inoculated with faeces will be investigated.

Phases 3 and 4: urban and rural communities. Ideally the research should involve collecting a stool sample from a random but age-stratified sample of people living in both urban and rural communities. Microscopy and the culture of stools in Robinson's medium would diagnose infections with E.histolytica while isoenzyme electrophoresis would disclose the carriage rate of potentially pathogenic zymodemes in the community.

However, conducting large scale field surveys simply to study E.histolytica would be expensive and difficult, so it is proposed either to join this research to another field project or to offer the diagnosis of E.histolytica as a service to proposed research. For example over the next 4 years a cohort study of cross-protection rotavirus and Shigella serotypes is planned as well as further vaccine trials. Such studies may provide material for research on E.histolytica or may provide the organisation in which research on E.histolytica could be performed. Supplementary funds would be sought for such work.

Timetable of research

Year 1 Establishment of isoenzyme electrophoresis. Establishment of hybridoma cells producing monoclonal antibodies (MAbs) in vitro. Preparation of reagents for testing amoebae for pathogenicity using MAbs. Establishment of E.histolytica in axenic culture. Development of indirect fluorescence antibody test and cellulose acetate precipitin test as diagnostic tests for amoebiasis.

Years 1-2 **Phase 1: an urban hospital.** Culture in Robinson's medium of about 2,500 stools collected as a part of the Dhaka Surveillance System and of about 350 - 400 stools found to contain amoebae. Blind testing of amoebae using both monoclonal antibodies and by isoenzyme electrophoresis will be done to distinguish between zymodemes.

Years 2-3 **Phase 2: a rural hospital.** Establish a sampling procedure at Matlab Treatment Centre to inoculate stools into Robinson's medium and send cultures found to contain amoebae to Dhaka for testing. Blind testing of amoebae isolated.
Phases 3 and 4: urban and rural communities. Establish a procedure to collect stool samples from an age-stratified sample of people living in urban and rural Bangladesh. Blind testing of amoebae isolated.

BIBLIOGRAPHY

Butler T, Islam M, Azad AK, Islam MR, Speelman P. (1987). Causes of death in diarrhoeal diseases after rehydration therapy: an autopsy study of 140 patients in Bangladesh. Bulletin of the World Health Organization 65: 317-323.

Diamond LS, Harlow DR, Cunnick CC (1978). A new medium for the axenic cultivation of Entamoeba histolytica and other Entamoeba. Transactions of the Royal Society of Tropical Medicine and Hygiene 72: 431 - 432.

Goldmeir D, Sargeaunt PG, Price AB et al. (1986). Is Entamoeba histolytica in homosexual men a pathogen? Lancet i: 641-644.

Hossain MM, Ljungstrom I, Glass RI, Lundin L, Stoll BJ & Huldt G (1983). Amoebiasis and Giardiasis in Bangladesh: parasitological and serological studies. Transactions of the Royal Society of Tropical Medicine and Hygiene 62: 552 - 554.

Mirelman D (1987). Effect of culture conditions and bacterial associates on the zymodemes of Entamoeba histolytica. Parasitology Today 3: 37 - 40.

Nanda R, Baveja V, Anand BS (1984). Entamoeba histolytica cyst passers: clinical features and outcome in untreated subjects. Lancet ii: 301-303.

Robinson GL (1968). The laboratory diagnosis of human parasitic amoebae. Transactions of the Royal Society of Tropical Medicine and Hygiene 62: 285 -294.

Ronsmans C, Bennish ML, Wierzba T. (1988). Diagnosis and management of dysentery by community health workers. Lancet ii: 552-555.

Sargeaunt PG (1987a). Zymodemes of Entamoeba histolytica. Parasitology Today 3: 158 - 159.

Sargeaunt PG (1987b). The reliability of Entamoeba histolytica zymodemes in clinical diagnosis. Parasitology Today 3: 40 - 43.

Sargeaunt PG & Williams JE (1978). Electrophoretic isoenzyme patterns of Entamoeba histolytica and Entamoeba coli. Transactions of the Royal Society of Tropical Medicine and Hygiene 72: 164 - 166.

Sargeaunt PG, Williams JE, Green JD (1978). The differentiation of invasive and non-invasive Entamoeba histolytica by isoenzyme electrophoresis. Transactions of the Royal Society of Tropical Medicine and Hygiene 72: 519 - 521.

Sepulveda B & Martinez-Palomo A (1985). Amebiasis. In: Tropical and Geographical Medicine, eds Warren KA & Mahmoud AAF. New York: McGraw- Hill

Stamm WP & Phillips EA (1977). A cellulose acetate membrane precipitin (CAP) test for amoebiasis. Transactions of the Royal Society of Tropical Medicine and Hygiene 71: 490 - 492.

Strachan WD, Chiodini PL, Spice WM, Moody AH, Ackers JP (1988). Immunological differentiation of pathogenic and non-pathogenic isolates of Entamoeba histolytica. Lancet i 561-562.

Wanke C, Butlet T, Islam M (1988). Epidemiologic and clinical features of invasive amebiasis in Bangladesh: a case-control comparison with other diarrheal diseases and postmortem findings. American Journal of Tropical Medicine and Hygiene 32: 335 - 341.

BUDGET FOR 3 YEAR PROJECT

	Year 1	Year 2	Year 3	Total
Personnel				
Coinvestigator (NOA)	8,616	9,908	11,394	29,918
Laboratory Technician (GS3)	3,864	4,444	5,110	13,418
Laboratory Technician (GS3)	3,720	4,278	4,920	12,918
Laboratory Assistant (GS1)	2,784	3,202	3,682	9,668
Operating costs				
Chemicals and media	5,000	5,000	5,000	15,000
Glassware and plastics	4,000	3,000	1,000	8,000
Photocopying and mimeography	500	500	500	1,500
Shipping	2,400	–	–	2,400
Hospital supplies	500	250	250	1,000
Cleaning supplies	100	100	100	300
Maintenance, telex, etc	350	350	350	1,050
Other costs	1,000	1,000	1,000	3,000
Capital Equipment				
Pharmacia flat electrophoresis bed and power supply	6,000			6,000
2 x Helena cellulose acetate electrophoresis apparatus	7,000			7,000
Objectives for inverted microscope	750			750
Jencons water still	750			750
Deioniser	1,200			1,200
Taylor-Wharton 35VHC cryobank and accessories	2,000			2,000
Travel	200	400	400	1,000
Computing				
	50,734	32,432	33,706	116,872

TOTAL OVER 3 YEARS US$ 116,872

Justification of budget

1. The cost of salaries for the staff required to undertake the work comes to $53484, 56% of the total.

2. Large volumes of media will be required each week in order to culture E.histolytica.

3. A flat aluminium electrophoresis bed will be required for starch and polyacrylamide electrophoresis in order to keep gels cool. A thermocirculator and cooler are already available.

4. One Helena cellulose acetate electrophoresis apparatus is already available, but if 3 enzymes are to be studied then three beds will be required to make the most efficient use of enzyme beads.

5. A supply of distilled deionised water will be needed in the Parasitology Laboratory.

6. The cryobank will be needed to store in liquid nitrogen isolates of E.histolytica.

Replies to comments on research proposal

"Establishment of techniques to identify pathogenic strains of *Entamoeba histolytica* for use in clinical and epidemiological studies in Bangladesh"

Dr. David Warhurst

His comments have been included and a more optimistic, revised version was written.

Dr. Ralph Neal

1. Morphological determination of species

Suggestion incorporated. Stained smears will be prepared.

2. Serology

Once isoenzyme electrophoresis has been fully established a research proposal may be written in which blood would be collected from asymptomatic cyst passers to detect antibodies. This would be submitted as a separate study.

3. Survey very in villages and towns

This point is recognised by the proposal already, but to set up a surveillance system to look only for *E.histolytica* when the prevalence may be no more than 10 - 15% would be extremely expensive. It would be more cost effective to examine stools provided during the course of other field research projects and such work will be included in forthcoming studies if it can be arranged.

4. Cloning

This will be necessary if more than one zymodeme is present in an isolate, and it will be done.

5. WHO definition of amoebiasis

This definition fails to recognise that the suffix "-iasis" refers to the signs and symptoms of disease. To use the term "amoebiasis" to indicate a state of infection as well as disease is unfortunate and imprecise.

6. Metronidazole

Who is the "he" in this sentence, Andrew Hall or Ralph Neal? I consider metronidazole to be a toxic drug and side effects are common. If Dr. Neal doesn't the he is in a minority.

Axenic culture

Point recognised, but I have considerable experience of lishing *Giardia* in axenic culture. If necessary we can n axenic cultures from other sources in order to provide ens.

Distinguishing *E.histolytica* from other amoebae

Staining will do this (see point 1).

Review of Research Proposal

Establishment of techniques to identify pathogenic strains of *Entamoeba histolytica* for use in clinical and epidemiological studies in Bangladesh.

by

Dr David Warhurst

Amoebiasis Unit
Hospital for Tropical Diseases
London NW1

and

Department of Parasitology
London School of Hygiene and Tropical Medicine
Keppel Street
London WC1E 7HT.

1. Reads well.

2. Page 2. Amoebiasis is probably diagnosed more in Mexico than in any other country rather than being more common.

3. Page 3. It is only common practice not to treat asymptomatic homosexuals in the UK as the chances of reinfection are high. The same is also true for India: ~~pathogenic zymodemes are uncommon and~~ the chances of reinfection are high, so asymptomatic infections are often left untreated.

4. Phase 2. Cultured for what?

5. The proposal should be more optimistic: it is necessary to know whether *E.histolytica* is important, not whether it is unimportant!

6. Since ~~If~~ little is known about *E.histolytica* in Bangladesh then the work will be useful and should be supported.

DC Warhurst.

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Department of Medical Parasitology

8 September 1989

By Fax: 010 880 2 411846

Dr Saul Tzipori
Associate Director
Laboratory Sciences Division
ICDDR,B
GPO Box 128
Dhaka - 1000
Bangladesh

Dear Saul

I enclose a copy of Dr R A Neal's comments on our proposal - I was quite pleased by them.

He made a few other comments on an informal basis:

1 Page 2 - the WHO definition of amoebiasis is simply the state of being infected, with or without symptoms;
2 Page 3 - he does not regard metronidazole as a toxic drug;
3 Page 5 - establishing new strains in axenic culture is not easy°
4 Page 7 - other amoeba such as E.coli also grow in Robinson's medium; perhaps we should include the preparation of Trichrome-stained smears from positive cultures in our protocol. These could then be examined and only those containing E.histolytica put in for zymodeme testing. What do you think?

I hope this is helpful, and that the project is soon funded.

With very best wishes to yourself, Andrew and the others.

Yours sincerely

John

J P Ackers
Senior Lecturer

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Department of Medical Parasitology

7 September 1989

RESEARCH PROPOSAL : A Hall, M Haque, J P Ackers
Establishment of techniques to identify pathogenic strains of E. histolytica for use in clinical and epidemiological studies in Bangladesh

Basic data on the incidence of E histolytica infection in Bangladesh is badly needed and this proposal should be able to provide some valuable information. All the techniques required are described. As far as isolation in culture is concerned there are other systems (e.g. Dobell & Laidlaw media) which are as good as the Robinson method and might be considered in initial work. More emphasis perhaps should be given to morphological determination of species grown since the Robinson system is not specific for E. histolytica.

Zymodeme analysis will provide a good opportunity to check the Sargeaunt theory. Failure to test for serum antibodies is a mistake though the difficulties are appreciated and understood.

If the survey is limited to hospital cases, the results will be biased and may not indicate a true local or national incidence. The authors are urged to link in with other surveys on a village or town basis if possible.

Whether the Sargeaunt observation on the association of certain zymodemes with pathogenicity is correct needs further confirmation which studies such as these may provide. It is hoped that studies on infectivity and virulence as indicated by lysis of tissue culture cells in vitro or by animal inoculation could be included in a future, or better still, parallel proposal.

One technique not mentioned in the proposal which may be valuable in analysis of zymodemes is of course cloning.

pp. Wendy Read
(Secretary)

Dr R A Neal
Winches Farm Laboratories
London School of Hygiene and Tropical Medicine