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ETHICAL REVIEW COMMITTEE, ICDDR,B.

Date 18.3.1990
19.3.1990

ipal Investigator Andrew Hall

cation No. 90-006

of Study Albendazole as a treatment

infections with Giardia.

Trainee Investigator (if any) _____

Supporting Agency (if Non-ICDDR,B) _____

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

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

urce of Population:
) Ill subjects — Yes No
) Non-ill subjects — (Yes) No
) Minors or persons under guardianship — (Yes) No

es the study involve:
) Physical risks to the subjects — Yes (No)
) Social Risks — Yes (No)
) Psychological risks to subjects — Yes (No)
Discomfort to subjects — Yes (No)
Invasion of privacy — Yes (No)
Disclosure of information damaging to subject or others — Yes (No)

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

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

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.

APR 19 1990

(PTO)

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

ncipal Investigator

Trainee

90-006
19.3.1990

INVESTIGATOR: Andrew Hall

CO-INVESTIGATOR: Qamrun Nahar

TITLE: Albendazole as a treatment for infections with *Giardia*

START DATE: 1st May 1990

END DATE: 30th April 1991

BUDGET REQUESTED: US$ 38,447

FUNDED BY:

HEAD OF PROGRAMME: Dilip Mahalanabis 19/3/90

AIMS

General aim
To study the effectiveness of albendazole as a treatment for infections with *Giardia intestinalis*

Specific aims
To compare metronidazole and albendazole as treatments for infections with *Giardia intestinalis*.

To compare albendazole given as a single dose with albendazole given as three single daily doses as treatment for infections with *Giardia*.

Significance
If intestinal parasitic infections are to be controlled then part of that control is likely to involve treatment of existing infections. There is a need for a single dose treatment which is effective not only against intestinal worms but also against important intestinal protozoa. Albendazole is effective against all the common intestinal nematodes of man, and against some trematodes and cestodes. An effect against *Giardia* would be an additional benefit. Regular treatment with a drug with such broad effects against intestinal parasites could help to reduce both the prevalence and intensity of infections and thus any effects on the health of the community, and will help to eliminate reservoirs of infection.

ETHICAL IMPLICATIONS

All the drugs to be used in this research are licensed and are on open sale in Bangladesh. There would be advantages for the treatment of infections with *Giardia* if a drug less toxic than metronidazole could be found, particularly if it was effective as a single dose. The subjects taking part in this research will receive treatment for intestinal parasitic infections and will have the services of a physician during the course of the study.

BACKGROUND

Intestinal parasitic infections are among some of the most common and ubiquitous infections of man. Recent estimates suggest for example, that 25% of the worlds' population are infected with *Ascaris lumbricoides* while *Giardia intestinalis* (= *G.lamblia*) is probably the world's most widespread intestinal protozoan: it is endemic in many developed countries as well as in most developing countries (Warren & Mahmoud, 1985).

At the moment at least two different drugs are needed to treat infections with both intestinal protozoa and intestinal nematodes. The drugs commonly used drugs to treat most species of intestinal protozoa are metronidazole and tinidazole: they are very similar chemically and are both active against *Giardia intestinalis* and *Entamoeba histolytica*. Metronidazole is the drug of choice to treat *Giardia* in the USA (AMA, 1987). Both metronidazole and tinidazole are associated with mild and transient side effects including nausea, abdominal pain, vomiting, a metallic taste, headache, urticaria and reversible neutropenia (Gustafsson *et al.*, 1987).

Mebendazole is probably the most commonly used drug with activity against the major intestinal nematodes of man, *Ascaris lumbricoides, Trichuris trichiura* and hookworms, and is usually prescribed as two doses a day for three days. Some other drugs, usually administered as a single dose such as pyrantel pamoate and levamisole, are highly effective against *Ascaris* but are less effective against *Trichuris* and hookworms. Finally, a relatively new drug, albendazole, has been shown to be effective when given as a single dose to treat *Ascaris, Trichuris* and hookworms (Pene *et al.*, 1982; Ramalingam *et al.*, 1983).

Albendazole is a benzimidazole derivative chemically related to mebendazole, thiabendazole and flubendazole. It is rapidly converted in the body to albendazole sulphoxide, the main metabolite with anthelmintic activity (Gustafsson *et al.*, 1987). A single dose of 400 mg of albendazole given to adults and children older than 2 years is recommended by the manufacturers as treatment for adult intestinal nematodes, including both species of hookworms, *Ascaris lumbricoides* and *Trichuris trichiura*. Using different regimens of the drug albendazole has also been shown to have an effect against tissue invasive nematodes such as *Strongyloides stercoralis* and hookworm larvae, against some adult trematodes, against cestodes of the genus *Taenia*, and is being studied as a possible treatment for hydatid disease (Anonymous, 1984). Albendazole appears to have an effect on a wider range of helminths than any other currently available drug, and it shows either good rates of cure or significant reductions in egg counts for the most common intestinal nematodes, even when given as a single dose (Pene *et al.*, 1982; Ramalingam *et al.*, 1983). One of these studies recommended that infections with *Trichuris* should be treated with 600 mg of albendazole (Pene *et al.*, 1982).

Recent research at Murdock University in Australia has shown that albendazole is active *in vitro* against *Giardia* and also that it appears to be more active than metronidazole or tinidazole (Thompson, personal communication of data in press). In these experiments, the lowest concentration of drug which had an effect on trophozoite motility, structure or adherence was defined as the minimum inhibitory concentration (MIC). The lowest concentration of drug at which no morphologically normal and dividing

trophozoites were observed after 7 days culture was defined as the minimum lethal concentration (MLC). After exposing trophozoites to the drug for only 4 h the following values were obtained:

	MIC	MLC
Metronidazole	2.6 µg/ml	> 20.5 µg/ml
Tinidazole	1.3 µg/ml	> 20.5 µg/ml
Albendazole	0.3 µg/ml	1.3 µg/ml

This table indicates that at the concentrations of drug used in the experiments, the trophozoites of *Giardia* exposed to metronidazole or tinidazole for 4 hours can recover whereas trophozoites exposed to albendazole do not. The concentration of albendazole which inhibited the growth of *Giardia* was nearly nine times lower than the concentration of metronidazole which had the same effect.

Because albendazole is also active against gut nematodes, if albendazole was found to be an effective treatment for *Giardia*, and particularly if it could be given as a single dose, then the drug could have great value as a part of parasite control programmes in circumstances where polyparasitism is common such as the slums of Dhaka.

Giardia in the slums of Dhaka

Infections with *Giardia* are common in the slums of Dhaka. In a recently finished study of *Ascaris* in a slum in Mirpur, the prevalence of infections with *Giardia* among 543 children aged between 5 and 10 years was nearly 12% (Hall, unpublished observations). In a prospective study of the acquisition of infections with *Giardia* conducted in another part of the same slums, the average number of infections diagnosed each month was 8.5 in a sample of about 200 children aged less than 3 (Hall, unpublished observations). During the 22 months of this study, during which stool samples were collected and examined once a month, only 87 (38%) of the 226 children studied were never infected with *Giardia*.

RESEARCH PLAN

The null hypothesis proposed is that albendazole, given either as a single dose or as three daily doses, is as effective as metronidazole in treating infections with *Giardia*. This hypothesis will be tested in an investigation to be undertaken in an urban slum in Dhaka.

Study site, sample and sample size

The study will be conducted in Milat Camp, Mirpur Section 11. The study entitled "*Giardia* and persistent diarrhoea" (Protocol number 86-021) was conducted in this area and the investigators and staff are well known to the local people. Mothers whose children took part in the previous study of *Giardia* will be asked to allow their children aged between 5 and 10 years old to take part in the proposed new investigation. Participation will be by informed consent. New families will be recruited if necessary.

The study sample will be children aged between 5 and 10 years old. The prevalence and intensity of parasitic infections is known to be high in this age group and for practical reasons such children are most amenable to study, particularly in terms of collecting stools. Each child will be given a medical examination by the project physician before being enrolled in order to ensure that there are no underlying medical conditions. During this examination or at any time during the study, children with medical complaints will be given either free treatment by the project physician or referred to a suitable medical facility.

The size of the sample to be chosen for study will depend on the need to be able to distinguish between treatments for *Giardia* in terms of their effectiveness, and can be calculated according to the following formula (Kirkwood, 1988):

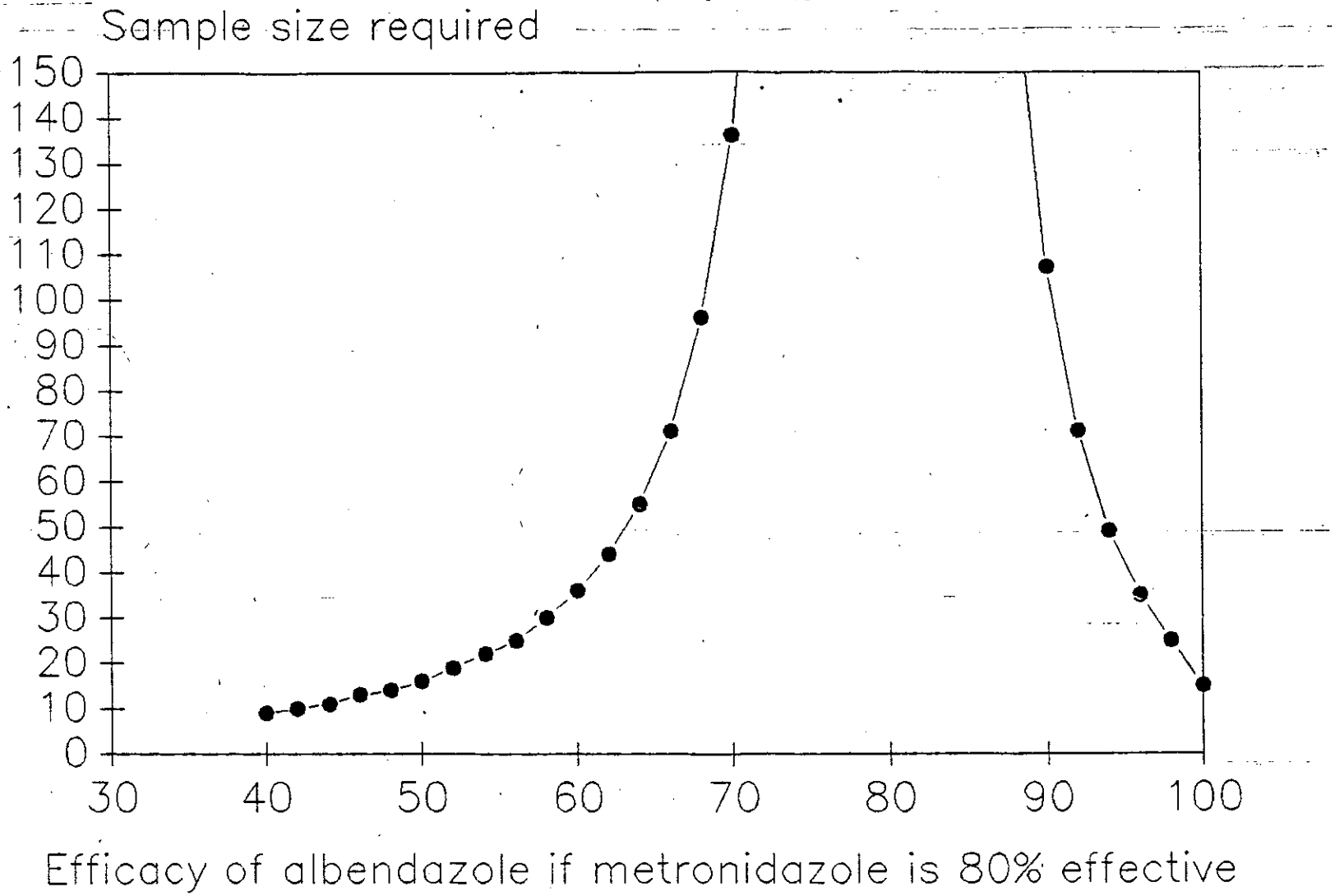
$$n > \frac{\{u \sqrt{[\pi(1-\pi)]} + v \sqrt{[\pi_o(1-\pi_o)]}\}^2}{(\pi-\pi_o)^2}$$

Using this formula it can be shown for example that if a sample size of 30 is taken for each group and if a 5 day course of metronidazole is assumed to treat 80% of infections (π_o = 0.8) with *Giardia* (WHO, 1981; Reynolds, 1989), using a power of 80% (u = 1.28) and at a 5% level of significance (v = 1.96) it would not be possible to conclude that another drug was more effective than metronidazole if it was less than 97% effective, or to say that it was less effective than metronidazole if it was more than 58% effective. In other words, if between 58% and 97% of cases are cured by another treatment then that drug is indistinguishable from metronidazole in its efficacy and the null hypothesis is accepted; if fewer or more cases are cured then the drug is less or more effective and the null hypothesis is rejected. From such a calculation it is obvious that as the sample size increases so does the ability to discriminate between treatments. By varying the proportion of people treated effectively by a test drug in the equation (π), a range of sample sizes can be calculated and plotted (see Figure). This plot shows that if two drugs are very similar in their efficacy, a very large sample size is required to be able to tell which is more effective. The sample size to be used will therefore be a compromise between the power to discriminate between drugs and practical considerations such as the number of cases likely to occur, the number of stools which can be examined daily by a limited number of staff using time consuming quantitative techniques, and the cost of the research.

For these reasons a sample size of 60 cases of *Giardia* per group has been chosen, so a total of 180 cases would be required to complete a study of three treatments. With a sample size of 60 per group there would thus be no significant difference in the efficacy of drugs if the test treatment was between 65% and 93% effective, assuming that metronidazole is 80% effective. If less than 65% or more than 93% of infections were cured then the test treatment would be significantly different compared with metronidazole.

With a prevalence of *Giardia* in the slums of Dhaka of about 12% and assuming a rate of infection of 4 cases per 100 children per month, then 400 children studied for 9 months should provide a sample sufficient to conduct the proposed study.

FIGURE


Sample size required
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
30
40
50
60
70
80
90
100
Efficacy of albendazole if metronidazole is 80% effective

Treatment of infections with *Giardia*

Infections with *Giardia* will be diagnosed by the microscopical examination of a direct smear of fresh faeces for the cysts and trophozoites of parasites, and a sample will also be fixed for examination by an ether concentration technique (Hall, 1981). If *Giardia* is diagnosed, information will be recorded about signs and symptoms of infection, about recent diarrhoea and about the number and type of stools passed in the last 24 hours. Treatment will be given for all infections, symptomatic or asymptomatic.

It is estimated that there will be between 40 and 50 subjects infected with *Giardia* at enrolment. These subjects will receive one of three treatments as follows: Group 1 will be given albendazole as a single dose of 600 mg; Group 2 will be given albendazole as a single daily dose of 400 mg for 3 days; Group 3 will be given metronidazole at a dose of 10 mg/kg body weight in three daily doses for 5 days. The drugs given to Groups 2 and 3 will be provided each day by fieldworkers in a bottle containing the daily dosage.

Because of the different doses and dosages it would be extremely difficult to conduct a double-blind study: the staff would be aware which drug is being given and it is likely that the subjects would find out as well, particularly if two children in the same household were assigned to receive different treatments. Nevertheless bias will be avoided for the following reasons. First, the order in which drugs are to be given will be decided before the study begins using accepted randomisation procedures (see below). Secondly, neither the project staff nor the mother can influence the outcome of the treatment given to a child as this will be determined on the basis of the presence or absence of *Giardia* in the faeces alone: the outcome of treatment will be evaluated simply in terms of whether a parasitological cure has been achieved or not. All stool samples will be sent from the Mirpur field site to the ICDDR,B by a messenger and will be identified only by a serial number — no names or ID numbers will be used. In this way the laboratory staff will not know whose sample is being examined so they cannot be influenced by the treatment given.

The process of assigning subjects for treatment will be performed as follows. Because there are no methods of quantifying the intensity of infections with intestinal protozoa, the subjects will be simply classed as infected or uninfected. Infected subjects will be randomly allocated to receive a treatment by selecting in serial order a numbered envelope containing the number of the treatment to be given. The number in each envelope, either 1, 2 or 3, will be generated by the random numbers routine in the statistical programme Statpac Gold (Walonick Associates, USA). Stratified randomisation was not recommended by one of the external reviewers of this proposal (S.M. Gore).

After the treatment has been completed, stool samples will be collected on days 3, 6 and 9 after the last or only dose of drug. As reinfection with *Giardia* could theoretically occur immediately after treatment, and as the median prepatent period for infections is about 14 days (Jokipii and Jokipii, 1977), stools collected on 3 occasions over the first 10 days after treatment should be sufficient to provide evidence of unsuccessful treatment. A direct smear of faeces will be examined for cysts and trophozoites before using a concentration technique to detect cysts. A sample of faeces will also be placed in phosphate buffered saline and stored in a freezer set at -20 °C.

This sample will be tested at a later date for *Giardia* antigen using an ELISA developed at the London School of Hygiene and Tropical Medicine (Green *et al.*, 1985; Miles, pers. comm.). Three diagnostic techniques will thus be used to determine whether treatments were successful. If treatment for *Giardia* is found to be unsuccessful a course of metronidazole will be given. If subjects are found to be infected with intestinal nematodes after treatment for *Giardia* they will be given a course of mebendazole.

When children become infected with *Giardia* during the study they will be assigned to receive a treatment as described above. If two or more children are diagnosed to be infected at the same time the envelope containing the number of the treatment they are to receive will be selected by stool serial number order: the lower or lowest stool serial number will be allocated treatment first.

After treatment, stool samples will then be collected once a month and screened for infections with *Giardia*. If children become reinfected with helminths they will be treated once more at the end of the study.

Analysis of results

The proportion of infections which are parasitologically cured by each test drug will be compared with the proportion successfully treated by the control. Comparisons will also be made between the test treatments.

REFERENCES

AMA (1987). *American Medical Association Drug Evaluations.* 6th edn. Philadelphia: W.B. Saunders

Anonymous (1984). Albendazole: worms and hydatid disease. *Lancet* II: 675 - 676.

Green, E.L., Miles M.A. and Warhurst, D.C. (1985). Immunodiagnostic detection of *Giardia* antigen in faeces by a rapid visual enzyme-linked immunosorbent assay. *Lancet* ii: 691 - 693.

Gustaffson, L.L., Beerman, B. and Abdi Y.A. (1987). *Handbook of Drugs for Tropical Parasitic Infections.* London: Taylor & Francis.

Hall A. (1981). Quantitative variability of nematode egg counts in faeces: a study among rural Kenyans. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 75: 682 - 687.

Jokipii A.M.M. and Jokipii L. (1977). Prepatency of *Giardia*. *Lancet* 1: 1095 - 1097.

Kirkwood B.R. (1988). *Essentials of Medical Statistics.* Oxford: Blackwell Scientific Publications.

Pene, P., Mojon, M., Garin, J.P., Coulard, J.P. and Rossignol J.F. (1982). Albendazole: a new broad spectrum anthelmintic. Double-blind multicenter clinical trial. *American Journal of Tropical Medicine and Hygiene* 31: 263 - 266.

Ramalingam, S., Sinniah, B. and Krishnan, U. (1983). Albendazole, an effective single dose, broad spectrum anthelmintic drug. *American Journal of Tropical Medicine and Hygiene* 32: 984 - 989.

Reynolds J.F. ed. (1989). *Martindale: The Extra Pharmacopoeia*, 29th ed. London: The Pharmaceutical Press.

Warren, K.S. and Mahmoud, A.A.F. eds (1985). *Tropical and Geographical Medicine.* New York: McGraw-Hill.

WHO (1981). Intestinal protozoan and helminthic infections. *WHO Technical Report Series No. 666.* Geneva: WHO.

PERSONNEL

Project Physician (Dr. Quamrun Nahar)	NO A - 2	9 months
Health Assistant (Nyati Sarker)	GS 3 - 2	9 months
Health Assistant (Nurjahan Akhter)	GS 3 - 4	9 months
Health Assistant (Salima Akter)	GS 3 - 4	9 months
Laboratory Technician (R.N. Sarker)	GS 3 - 4	9 months
Laboratory Technician (New post)	GS 3 - 1	9 months
Caretaker/Cleaner (A. H. Howlader)	GS 1 - 1	9 months
Data entry technician (Temporary post)	GS 3 - 5	1 month

BUDGET

	US$
Personnel	
Project Physician	7,100
Health Assistant	3,175
Health Assistant	3,175
Health Assistant	2,950
Laboratory Technician	3,175
Laboratory Technician	2,720
Caretaker/Cleaner	600
Data Entry Technician	350
	23,245
Supplies and Material	
Drugs	2,000
Glassware	100
Hospital supplies	200
Stationery	200
Chemicals	500
Janitorial supplies	150
Non-stock items	1,500
	4,650
Other costs	
Rent	600
Interdepartmental services	
Transport, Dhaka	200
Xerox, mimeography	300
Telex	100
Staff clinic	150
Tests	100
	850
TOTAL	29,345
Overheads (31%)	9,097
GRAND TOTAL US$	38,442

"সম্মতি পত্র"

যে সমস্ত বাচ্চাদের বয়স ৫ থেকে ১৫ মাসের মধ্যে এবং এই গবেষণা কার্যে অংশগ্রহণ করিতে তাহাদের অভিভাবকগণকে এই সম্মতিপত্র পড়ে শোনানো হবে এবং অভিভাবকগণ সম্মতিপত্রে স্বাক্ষরদান করিবেন। গবেষণার বিষয় বস্তু—"অন্ত্রিক প্যারাসাইটের জন্যে এক ডোজের চিকিৎসা"।

শিশুর নাম:

১. বাচ্চার নাম:

২. বাচ্চার নাম:

আমরা আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র (কলেরা হাসপাতাল) মহাখালী, বাংলাদেশ থেকে এসেছি। আমরা কৃমিজনিত রোগের বিভিন্ন চিকিৎসার উপর গবেষণা করছি। যে সমস্ত ঔষধ আমরা ব্যবহার করছি তাহা বাংলাদেশে ব্যবহারের জন্যে অনুমতি প্রাপ্ত।

যদি আপনি আপনার ছেলেমেয়েদের এই গবেষণাকার্যে অংশগ্রহণ করিতে সম্মতিদান করেন তাহলে আমরা আপনার প্রত্যেক বাচ্চার নিকট হইতে একটি পায়খানার নমুনা সংগ্রহ করিব সংক্রামক রোগ পরীক্ষা করার জন্য। আমাদের চিকিৎসক আপনার বাচ্চাকে আমাদের চিকিৎসাকেন্দ্রে পরীক্ষা করিবেন।

যদি আমরা আপনার বাচ্চাকে সংক্রামিত দেখি তাহলে আমরা বিনামূল্যে চিকিৎসা করিব। চিকিৎসার পর আপনাকে অনুরোধ করা হইবে ২০শে হইতে ৩০শে পায়খানার নমুনা সংগ্রহ করিতে যাহা ঔষধের সফলতার জন্য পরীক্ষা করা হইবে। বাচ্চাকে পুনরায় চিকিৎসার দরকার হইতে পারে, এখন আমরা আপনার বাচ্চার নিকট হইতে প্রতিমাসে একটি করিয়া পায়খানার নমুনা সংগ্রহ করিব, আপনার বাচ্চা কৃমিজনিত দ্বারা আক্রান্ত হয়েছে কি-না তাহার পরীক্ষার জন্য। যদি আক্রান্ত হয় - তাহলে আমরা বিনামূল্যে চিকিৎসা করিব এবং ঐ ঔষধে কাজ হয়েছে কি-না তাহা দেখার জন্যে আপনার বাচ্চার পায়খানার নমুনা আবার সংগ্রহ করা হইবে। যদি ঐ ঔষধে কাজ না করে থাকে, তাহলে আমরা অন্য ঔষধ দিব।

আমাদের কাজের সময় খেয়াল রাখবেন যে সমস্ত পায়খানার নমুনাগুলি আমরা সংগ্রহ করছি তাহা আমাদের যেন ঐ বাচ্চার হয় যে এই গবেষণাকার্যে অংশগ্রহণ করেছে।

যে সমস্ত বাচ্চা এই গবেষণা কার্যে অংশগ্রহণ করছে তাহাদের জন্যে চিকিৎসাকেন্দ্র প্রত্যেকদিন বিকাল ২টা থেকে ৪টা পর্যন্ত খোলা থাকবে। যদি আপনার বাচ্চার পাতলা পায়খানা বা ডায়রিয়া হয়ে থাকে তাহলে তাহার পায়খানার নমুনা দয়াকরে আমাদের চিকিৎসাকেন্দ্রে নিয়ে আসবেন। সমস্ত চিকিৎসা এবং ঔষধ এই গবেষণাকার্যে অংশগ্রহণকারী বাচ্চাদেরকে বিনামূল্যে প্রদান করা হইবে। আপনি আপনার বাচ্চাকে যে কোন সময়

CONSENT FORM

(Translated version to be read to and signed by the Guardian of any child aged between 5 and 10 years of age taking part in the study entitled "Single dose treatments for intestinal parasites".)

Mother's name: _____

1. Child's name: _____

2. Child's name: _____

We are from the International Centre for Diarrhoeal Disease Research, Bangladesh (Cholera Hospital) in Mohakhali. We are studying different treatments for intestinal parasites such as *Ascaris* (*boro krimi*) and *Giardia*. All the drugs we are testing are licensed for use in Bangladesh.

If you agree to let your child or children aged between 5 and 10 years old take part we would like to collect a stool sample from each child and examine it for evidence of infection. Our physician will also give your child a medical examination at our treatment centre.

If we find that your child is infected we will then provided free treatment for the infection and will ask you to collect between one and three stool samples afterwards treatment to check that the medicine has worked. It may be necessary to treat the child again.

We would then like to collect a stool sample each month to check that your child has not become infected with *Giardia*. If it does become infected we will provide free treatment and will then need to collect three stool samples to check whether the drugs has worked. If the medicine hasn't we will provide another one.

During our work please make sure that the stool samples we ask for come only from the child we are studying.

A treatment centre will be operated between 2 pm and 4 pm each day for those children taking part in our study. If your child has loose stools or diarrhoea, please bring along a faecal sample to this clinic. All medical examinations and medicines will be provided free of charge to your children taking part in this study. You may withdraw your child from this study at any time.