chment 1.
CE SHEET)

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Date 5th August 1990
20.8.90

ncipal Investigator Andrew Hall

Trainee Investigator (if any)

ication No. 90-013 (Revised)

Supporting Agency (if Non-ICDDR,B) O.D.A.

e of Study A comparison of repeatedly n-wormy and repeatedly wormy

ildren infected with Ascaris

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

le 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

oes 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)

es 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

e 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.

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

ncipal Investigator

Trainee

20.8.90

INVESTIGATOR: Andrew Hall

COINVESTIGATORS: Kazi Selim Anwar, Tasnim Azim, K.M.A. Aziz, Rashidul Haque and M.A. Wahed.

TITLE: A comparison of repeatedly non-wormy and repeatedly wormy children infected with *Ascaris*

START DATE: 23rd October 1990

END DATE: 22nd July 1991

BUDGET REQUESTED: US$ 23,713

FUNDED BY: British Overseas Development Administration

DIVISION HEAD: Dr. A.K.M. Siddique

AIMS

To identify factors associated with a predisposition to heavy infections with *Ascaris lumbricoides*.

SIGNIFICANCE

The distribution of parasites in a human community tends to be highly aggregated, an observation which suggests a difference between people in their susceptibility to infection. If the majority of parasites are clumped in a few hosts, those hosts are more likely to suffer from ill health. A study conducted in Mirpur has indicated that reinfection with *Ascaris lumbricoides* after treatment is not random and that certain people are repeatedly heavily infected while others always remain lightly infected. The study proposed here is intended to shed light on the factors associated with this phenomenon in children identified to be repeatedly wormy.

ETHICAL IMPLICATIONS

A single venous blood sample is required in order to do this research because an important component of the study is an assessment of immune function. At least 5 ml of blood is required if sufficient mononuclear cells are to be obtained for phenotyping and proliferation tests. The subjects have willingly taken part in a research study on evidence for a predisposition to worminess for nearly two years, and have received the benefits of regular deworming, biweekly visits by health personnel and free treatment for medical problems. These services will continue during the investigation. Remuneration of subjects for collecting their worms with an approved amount of money (Tk 15 per person) will continue as before.

BACKGROUND

There is a growing awareness that the distribution of worms within a community of hosts is neither uniform nor random: their distribution tends to be aggregated so that most hosts harbour few parasites while a few hosts harbour large numbers of parasites (Anderson & May, 1985; Anderson, 1988). This distribution has important implications for the study of the impact of parasites on health and for the control of their transmission. An example of heterogeneity in the dispersion of worms is presented in the Figure, which shows the distribution of the numbers of *Ascaris* per person among 1,765 people living in a slum in Mirpur (Hall, unpublished observations). Of the 29,533 worms recovered after treatment with a drug which paralyses worms so that they are expelled intact, 71% of worms were excreted by only 30% of people. A good empirical model of this aggregated pattern of parasite numbers per host is the negative binomial distribution which is defined by two parameters, the mean (M) and a parameter k, which varies inversely with the degree of aggregation. M and k are related to the prevalence (p) by the following equation (Anderson, 1982):

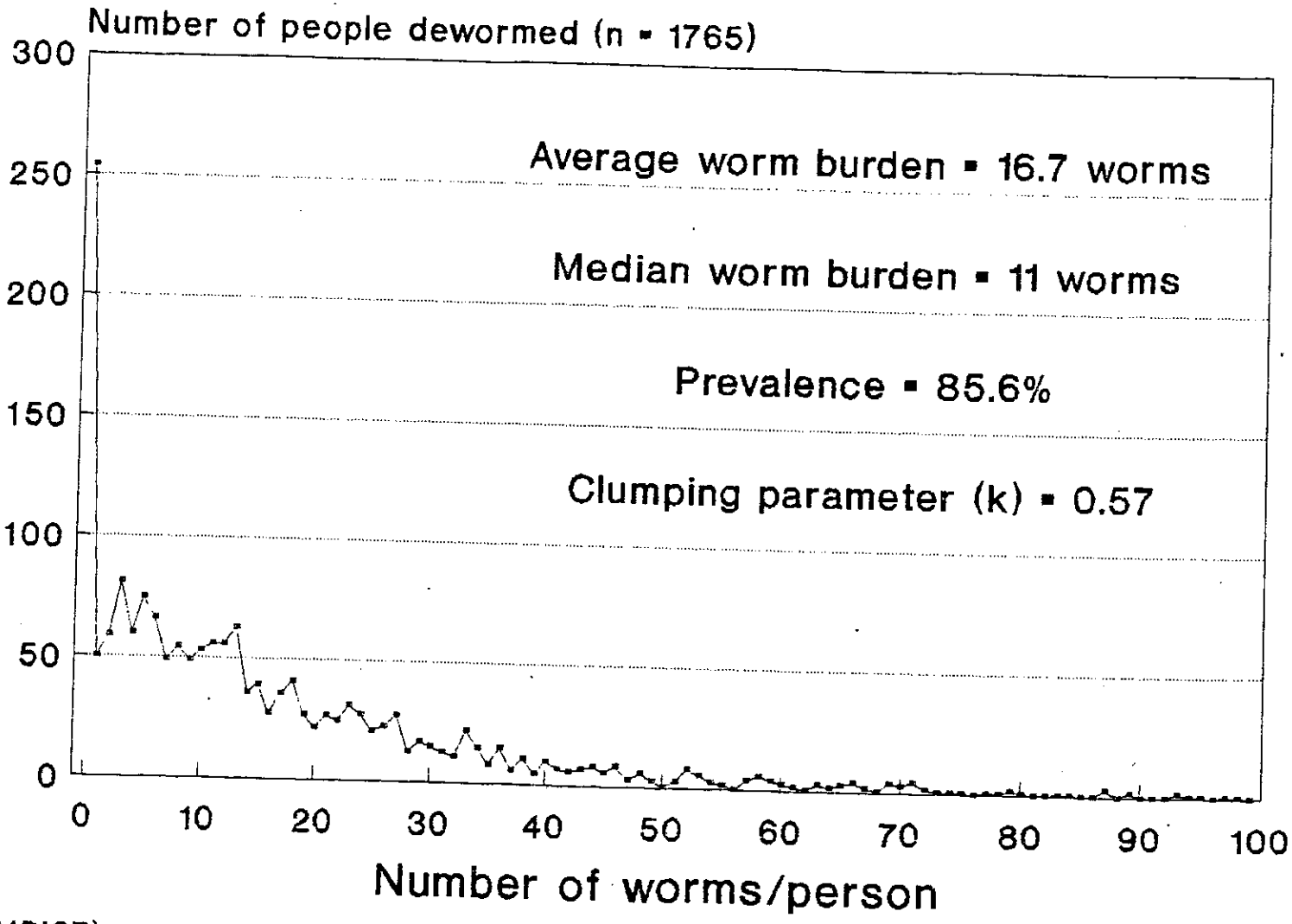
$$p = 1 - [1 + M/k]^{-k}$$

Values of k tend to range between 0.2 and 0.8 for helminth parasites and indicate that the host population is highly heterogeneous in its susceptibility to infection. The reasons for this heterogeneity are not understood and may be explained by a number of factors including the duration of exposure to infection, by the density of infective stages, by behaviour which increases exposure to infection,,and by non-specific and specific host responses to infection which may in turn be influenced by nutritional, physiological or genetic factors. It is the aim of the study described here to examine some factors which might be related to repeatedly heavy infections with *Ascaris*.

If only a proportion of people are heavily infected, with figures ranging from 5% to 30% depending on the local prevalence of infection, then any effects on health such as impaired growth are also likely be unevenly distributed within a group of infected people. It may seem obvious that heavily infected people are likely to be more severely affected than lightly infected people, but it has rarely been taken accurately into account when studying the nutritional consequences of parasitic infections, and this may explain the equivocal nature of such research so far (Schultz, 1982). Most investigations have used egg counts as an indicator of the intensity of infection, but for individuals they are a poor predictor of the actual worm burden (Hall, 1981). If possible it is important to collect worms expelled after anthelmintic treatment.

The recently finished study mentioned above of the intensity reinfection with *Ascaris lumbricoides* after treatment in which all worms were collected after treatment, has shown that reinfection is significantly non-random: a significant number of heavily infected people became heavily reinfected while a significant number of lightly infected people became lightly reinfected (Hall, unpublished observations from research proposal No. 86-035 originally entitled "*Ascaris* and wormy people" but renamed "Is there evidence for a predisposition to infection with *Ascaris*?").

DISTRIBUTION OF WORMS/PERSON



(WRMDIST)

In this preparatory study, a total of 1,017 people living in an urban slum in Mirpur were dewormed satisfactorily on three occasions six months apart. All worms expelled for 48 hours after treatment with pyrantel pamoate were collected in buckets. The worms were recovered, washed, sexed, counted and weighed, individually and in total. Burdens were classified as "light" if less than 15 worms were recovered or "heavy" if 15 or more worms were recovered (Anderson & Medley, 1985). The two contingency tables shown below (Tables 1 and 2) compare the proportions of burdens classified as heavy or light between rounds of treatment (n = 1016) and provide evidence that the intensity of reinfection after treatment is non-random. Although some people changed in classification between rounds of treatment, 501 people remained in the same classification and were either repeatedly heavily or repeatedly lightly infected.

TABLE 1.
A comparison of worm burdens in Round 1 against Round 2

		ROUND 1		
		Light	Heavy	
ROUND 2	Light	447	239	686
	Heavy	121	210	331
		568	449	1017

Chi squared = 74.3 (with Yates' correction), $P < 0.0001$

TABLE 2.
A comparison of worm burdens in Round 2 against Round 3

		ROUND 2		
		Light	Heavy	
ROUND 3	Light	585	172	757
	Heavy	101	159	260
		686	331	1017

Chi squared = 128.7 (with Yates' correction), $P < 0.0001$

As a part of the investigation just finished, the effect on the growth of children of regular deworming was also studied. At the end of April 1990 data on worm burdens and growth was complete for 203 children aged between 5 and 12 years old. Some basic information about the worm burdens of these children is presented in Table 3. This shows that the prevalence of

infections is high and that rate of reinfection after treatment with a drug known to be highly effective against *Ascaris* is also high (Gustaffson *et al.*, 1988).

TABLE 3.
The worm burdens of 203 children, aged 4 to 10 years at enrolment, on three occasions six months apart.

	ROUND 1	ROUND 2	ROUND 3
Number infected	187	176	187
Percentage infected	92%	87%	92%
Total no. of worms	4287	4532	3084
Mean no. of worms	21	22	15
Total weight of worms (kg)	9.132	9.169	6.224
Mean weight of worms (g)	45.0	45.2	30.7

Aim

The aim of the study proposed is to examine factors which might be associated with repeatedly heavy infections. Because the aggregation of intestinal helminths appears to be independent of age, gender and ethnic origin, particular emphasis will be placed on immunological factors associated with non-specific responses to infection, on tests which might reflect the strength of acquired immunity, and on deficiencies of nutrients which may impair an immune response. Environmental contamination and behavioural and hygienic factors will also be examined. Although the study has a retrospective element in that the history of the subjects' worm burdens is known, like most epidemiological studies the planned investigation cannot ascribe causal links, it can only indicate associations.

METHODS

Subjects

The number of children who can be studied is limited to those who have been satisfactorily dewormed on three occasions between August 1988 and February 1990 and who are still available for study. In April 1990 a total of 203 children fulfilled these criteria. In these 203 children reinfection with heavy or light worm burdens was significantly non-random and there were significant correlations between worm burdens in the different rounds of treatment.

The children have been ranked according to their average worm burdens and divided into three approximately equal groups: light (0 - 10 worms; n = 69), moderate (11 - 23 worms; n = 65) and heavy (≥ 24 worms; n = 69). All children in the light and heavy groups will be recruited for this investigation (n = 138). The children in the light group contain 96% of all children who were repeatedly lightly infected (n = 47) and the children in the heavy group contain 93% of all repeatedly heavily infected children (n=41). There were no statistically significant differences between the average age of these two groups of children in April 1990 or in terms of their nutritional status and both light and heavy groups had 34 females and 35 males. The

adequacy of this sample size will be examined later but the large number will allow for children whose parents refuse to take part.

All samples will be collected and tested blindly: no mother, no subject, no technician and none of the coinvestigators undertaking specialised tests, conducting interviews or making behavioural observations will know which is a repeatedly wormy child and which child is not. They will only be provided with a list of children in serial number order who are to be studied.

All 138 children will be visited at the start of the investigation and their parents will be asked if they are willing to let their children take part (see attached consent forms in English and Bangla). Mothers will be asked if they have treated their children for worms since the last treatment given by project staff.

Treatment for worms

Many of the methods used in the earlier study will be used in this investigation and are only described here briefly. A faecal sample will be collected from each child in order to estimate the concentration of helminth eggs in stools. Each child will then be given a dose of pyrantel pamoate syrup (Combantrin; Pfizer, Bangladesh) at 11 mg/kg body weight. All worms will be collected in buckets for 48 hours after treatment. The worms will be recovered, washed, sexed, and then weighed individually and in total to the nearest 0.1 g. All children will be studied whether they collect all their worms satisfactorily or not and the while the number of worms they pass will not change their classification.

About 7 days later each child will be given a single dose of 600 mg of albendazole in order to expel any species of worms not treated by pyrantel pamoate such as *Trichuris trichiura*. Another faecal sample will be collected a week later to check that egg counts have been significantly reduced and another dose of albendazole may be given if necessary. The same amount of money provided during the previous study (Tk 15) will be offered to the mother as remuneration for her time spent supervising her child and collecting all its stools.

Medical examination

Only children with no history of cough, fever or diarrhoea within the last month will be included in the study. At least four weeks after deworming each mother will be asked to bring her child or children to the project field office in Mirpur (House 13, Row 2, Block C, Section 11). This interval will allow children time to recover from the effects of worms acquired since they were last dewormed. Each child will be weighed and its height, mid-upper arm circumference and triceps skinfold thickness will be measured. Each child will be given a medical examination by the Project Physician and any current illnesses or complaints will be treated or, if necessary, referred to specialised medical facilities. A local anaesthetic cream will be applied to each childs arm to (EMLA; Astra Pharmaceuticals, U.K.) to eliminate or greatly reduce pain during venepuncture (Hellgren *et al.*, 1990). A 5ml sample of venous blood will be taken using a sterile "Vacutainer" tube (Becton Dickinson, USA) coated with heparin and the sample will be transported on ice to the Immunology laboratory of the ICDDR for processing and subsequent tests (see below). A treatment centre will be provided during the course of the study for all children taking part in this investigation.

Home hygiene and maternal knowledge
At the start of the investigation the current composition of the household will be recorded by age, gender and relationships. The presence in the family home of the following will be recorded: a water supply, a concrete floor to the house and/or compound and a latrine. The details to be recorded are listed Part 1 of the attachment.

At a later date the mother will be visited at home at a convenient time and asked questions about herself and her family circumstances including the use of latrines and sources of water, about her knowledge and practice of hygiene, and about her knowledge of *Ascaris*, its transmission and symptoms. The questions to be asked are listed in Part 2 of the attachment. Observations of the behaviour of children which might expose them to the eggs of *Ascaris* will be made. (K.M.A. Aziz).

All children under study, will be asked to wash their hands in a plastic bag of filtered water containing a few drops of liquid soap. The hands will be rinsed by water squirted from a bottle. The water will be centrifuged and examined microscopically for the eggs of *Ascaris*. This process will be repeated on two more occasions.

Infections of other members of household
All other members of the household will be asked to provide faecal samples for quantitative analysis. They will be treated with pyrantel pamoate at the recommended dose and will be asked to collect all their stools in buckets for the next 48 hours in order to quantify the worm burden. Treatment will be offered irrespective of whether they are willing to collect their stools or not. Remuneration will be given to those people who collect all their stools.

Environmental contamination with *Ascaris*
Samples of soil and household dust will collected and examined for the eggs of *Ascaris* according to the methods of Muller *et al.* (1989). A sample of 20g of soil will be scraped from an area 10 cm square from the centre of the compound of each household with an earthen floor and from the edge of the compound furthest from both the entrance to the compound and the entrance to the house. A small rechargeable, portable vacuum cleaner will be used to clean the whole surface area of the compound on a dry day. The dust collected will be weighed and examined for *Ascaris* eggs. Household compounds with cement floors will only be vacuumed.

Tests of immunological function
Cell mediated immune responses will be assessed using the CMI Multitest (Institut Mérieux). This involves the intradermal inoculation of a glycerin control and seven antigens: tetanus toxoid, diphtheria toxoid, *Streptococcus* Group C antigen, Tuberculin, *Candida albicans* antigen, *Trichophyton mentagrophytes* antigen and *Proteus mirabilis* antigen. The tests will be applied at the same time the blood sample is taken and the response will be evaluated 48 hours later when an induration of 2 mm in diameter will be counted as a positive reaction (Kazi Selim Anwar).

Venous blood will be sampled aseptically to provide blood for a total and differential cell count and for ABO and Rhesus typing in the Clinical Pathology Laboratory. The blood will be centrifuged and the plasma transferred in volumes of about 200 µl to Eppendorf tubes and then stored in a freezer at -20 °C until required. Mononuclear cells will be obtained by

centrifugation on Ficol-Paque and phenotyped by indirect immunofluorescence to determine the proportions of cells bearing the following markers: CD3 (T cells), CD4 (T-helper cells), CD8 (T-suppressor/ cytotoxic cells), CD20 (B cells) and CD25 (Interleukin-2 receptor bearing cells). The functional response of T-cells will be tested by assessing their proliferation in response to concanavalin A, B-cells will be stimulated with pokeweed mitogen , and both B- and T-cells will be stimulated with *Ascaris* antigen (Tasnim Azim).

The concentration in plasma of IgG, IgM, IgA and IgE will be estimated by assays set up on a COBAS BIO Centrifugal analyser using Dakopatts (Denmarke) antisera and controls (M.A. Wahed).

The eggs of *Ascaris* and *Trichuris* will be embryonated in 1% formalin and then hatched, killed, sonicated and centrifuged to provide crude larval antigens using methods described for *Toxocara* (Sugane & Oshima, 1984). The antigen will be used to develop an indirect enzyme-linked immunosorbent assay to detect antibodies to *Ascaris* in plasma. Control positive plasma will be prepared by injecting rabbits with larval antigens and Freund's complete adjuvant. The titre of antibodies in plasma will be estimated by serial dilution. One of the investigators has used such techniques to develop an ELISA to *Toxocara* antigens (Rashidul Haque).

One of the external reviewers of this proposal, Dr. Don Bundy of the Parasite Epidemiology Unit at Imperial College, London University, has offered to provide assistance to test plasma for cross-reacting antibodies to *Trichuris trichiura* and may be able to assist in testing plasma for antibodies to purified *Ascaris* antigens. Dr. Bundy and Professor Roy Anderson have established a Seroepidemiology Laboratory and their staff have over the last 3 years developed purified antigens of many important helminths. The P.I. will visit Dr. Bundy's laboratory in September to discuss means to test samples in the light of the views of the RRC and will report to the RRC the outcome of discussions on ways and means to conduct these specialised tests.

Nutritional biochemistry

Samples of plasma will be tested using standard methods for the following: for vitamin A using a high-performance liquid chromatograph; for retinol binding protein by single radial immunodiffusion; for zinc and copper by atomic absorption spectrophotometry; for iron using an assay for a COBAS Centrifugal Analyser; and for ferritin using an ELISA. All of these tests are offered by the Biochemistry and Nutrition Laboratory of the Centre and will be performed in duplicate with controls (M.A. Wahed).

Nutritional anthropometry and health

All the children who will be asked to take part in this study were recruited between 30th July and 24th December 1988. By the date of the intended start of the investigation, data on their growth and health will be available for nearly two years. It includes weight, mid-upper arm circumference and triceps skinfold thickness measured every month and height measured every three months. A biweekly diary of children's health has also been maintained.

Adequacy of sample size

There is little data available about normal values and standard deviations of the variables being measured (Table 4) for poor Bangladeshi children. However, using a formula for calculating a sample size where the aim is to compare two means (Kirkwood, 1988), estimates can be made for some variables

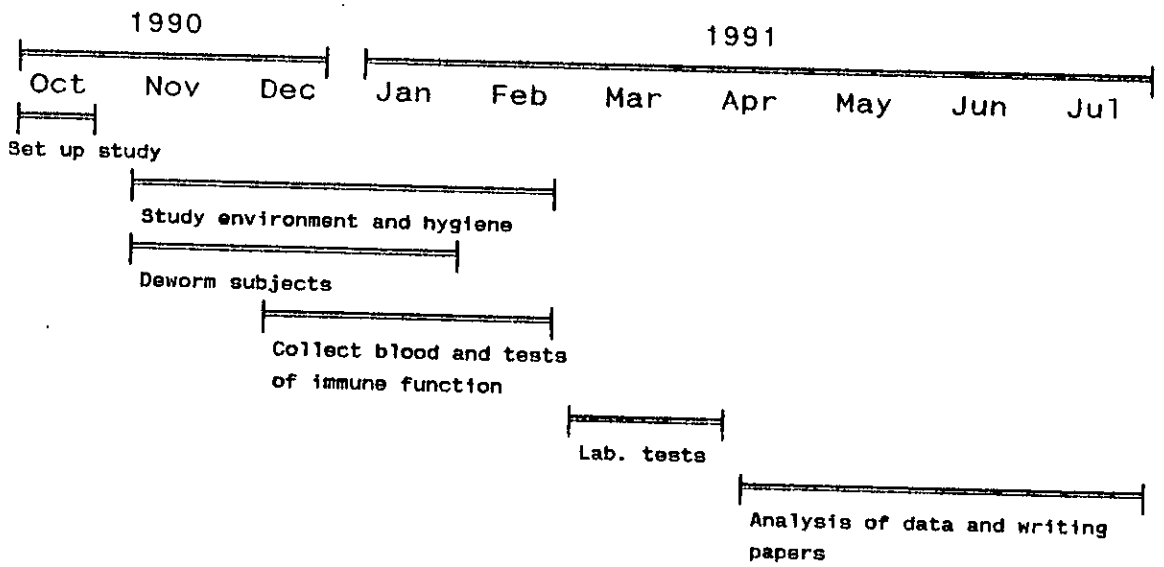
If it is assumed that the variances of values for each group are the same. Three representative variables will be examined: vitamin A, zinc and mononuclear cell proliferation.

Taking a mean plasma concentration of vitamin A of 20 µg/dl (S.D. 4 µg/dl), in order to show a 20% difference between groups (µ1 - µ2 = 4 µg/ml), with a power of 90% and at the 5% level of significance, a sample size of 21 per group is required. If the sample size eventually studied was 69 children per group a difference between means of 2.2 µg/dl would be significant, about 10% of the mean.

Taking a mean plasma zinc concentration of 0.86 mg/l (S.D. 0.14 mg/l) a 15% difference between groups would require a sample size of 25. With a maximum sample size of 69 per group a difference in group means of 0.08 mg/l would reach statistical significance, about 9% of the mean.

Estimates of cellular immune function tend to have larger variances than biochemical tests. Mononuclear cell proliferation is measured by the uptake of radioactive thymidine. A mean of 60,000 counts per minute (cpm) with an S.D. of 35,000 would require a sample size of 21 if a difference in means of 20,000 cpm was required. With a sample size of 69 children per group a difference of 11,100 cpm would be significant, about 20% of the mean.

Plan of research



Analysis of data

Much of the data concerning worms, such as egg counts and the number of worms recovered, is not normally distributed and is best described by the negative binomial distribution. All data will be tested for normality before statistical tests are applied and will be transformed if necessary to remove skewness.

Each variable listed in Table 4 will be compared between repeatedly lightly infected and repeatedly heavily infected children using t-tests for data which is normally distributed and using Wilcoxon's rank sum test for data which is not normally distributed and cannot be transformed. If

significant differences are found then a multiple regression analysis will be performed on the data in which the possible confounding variables of age and nutritional status will be examined. The relationship between all significant variables will also be examined. If the relationship between the dependent variable and an explanatory variable (x) is non-linear then it will be redefined into distinct subgroups and included as a factor, or an algebraic description of the relationship (x^2, x^3) may be sought and included into the equation (Kirkwood, 1988).

Table 4

Variables to be studied for children taking part in the investigation with the units or values of each.

Worm burden	
Average number of worms	Mean worm burden
Average weight of worms	Mean weight of burdens
Environmental contamination	
Concentration of eggs in soil and household dust	Eggs/gram
Immunological tests	
CMI Multitest	Diameter of indurations
Total leucocytes	Nos/ml of blood
Total T-cells (CD3)	Proportion of mononuclear cells
T-helper cells (CD4)	Proportion of T-cells
T-suppressor cells (CD8)	Proportion of T-cells
Total B-cells (CD20)	Proportion of mononuclear cells
Lymphocyte proliferation tests	
T-cells with con-A	{Stimulation indices or
B-cells with pokeweed	counts per minute}
Immunoglobulin classes	
IgA	g/l plasma
IgG	g/l plasma
IgM	g/l plasma
IgE	mg/l plasma
Ascaris antibodies	
ELISA using crude *Ascaris* antigen	Titre
Nutritional biochemistry	
Vitamin A	µg/100 ml
Retinol binding protein	mg/100 ml
Zinc	µmol/l
Copper	µmol/l
Iron	µmol/l
Ferritin	µg/l
Genetic markers	
ABO and Rhesus blood groups	

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PERSONNEL

Project Physician (Dr. Kazi Selim Anwar)	NO A	4 months
Health Assistant (Md. Shaihdul Alam)	GS 3 - 3	4 months
Health Assistant (Rifat Sultana)	GS 3 - 3	4 months
Laboratory Technician (Md. Lutfar Rahman)	GS 3 - 3	4 months
Laboratory Attendant (Majed Ali)	GS 1 - 3	4 months
Field Attendant (Syful Alam Miah)	GS 1 - 3	4 months
Field Attendant (A.K.M. Shaihdul Islam)	GS 1 - 3	4 months
Field Helper (Badrunessa Dulari)	Daily pd	4 months
Field Helper (Noorjahan Baby)	Daily pd	4 months
Caretaker/cleaner (Md Shahid)	Daily pd	4 months
Laboratory Technician	GS 5 - 3	1 month
Laboratory Technician	GS 5 - 3	1 month

BUDGET

An application for funds totalling £39,314 (approx. US$63,000) has been submitted to the British Overseas Development Administration and contains a request for funds for supporting Andrew Hall as well as for approximately $8,000 to buy reagents and materials in London for conducting many of the tests to be done at the ICDDR. The ODA allows overheads to be charged on "salary emoluments only".

	US$
Personnel	
Project Physician	2,872
Health Assistant	1,096
Health Assistant	1,096
Laboratory Technician	1,096
Laboratory Attendant	966
Field Attendant	966
Field Attendant	966
Field Helper	180
Field Helper	180
Caretaker/cleaner	180
Laboratory Technician	500
Laboratory Technician	500
ICDDR,B overheads on salaries	3,285
Supplies and Materials	
Drugs	800
Lab. supplies	1,000
Office and janitorial supplies	200
Xerox, mimeography, publication	800
Glassware	400
Hospital supplies	400
Petrol for local transport	400
Repairs, servicing	300
Interdepartmental services	
Biochemical and Clinical Pathology Tests	2,500
Telex	300
I.V. fluids	80
Other costs	
Other contractual services	2,400
Remuneration of subjects	250
US$	23,713

CONSENT FORM

HOUSEHOLD ID:
CHILD'S NAME:
MOTHER'S NAME:

As you know, we have been measuring the growth of your child for almost two years now. We have also collected all your child's worms on three occasions and have done the same for over 1,000 people in your community. We have discovered that some people always seem to have many worms inside them while others have few worms. For this reason we would like your permission to involve your child in some research to try to find out why some children always have many worms. To do this we want to do medical tests on a number of children who repeatedly have lots of worms and compare them with children who usually have very few worms.

First we would like to collect a stool sample from your child to test and then give medicine to expel worms as before. We would like you to collect all your child's stools in buckets for 48 hours as you did before so we can see how many worms are passed. We would also like to collect stools from the other members of your household, give you all medicine for worms and ask you to collect your stools in buckets after treatment in the same way. As on previous occasions we will provide remuneration for collecting worms after treatment of Tk 15 per person.

When you are free we would like to spend some time to ask you a few questions about yourself and about your family. We would also like to collect some samples of soil and dust from in and around your house to see if we can find the eggs of *Ascaris*.

About a month after we have treated your child we would like our doctor to give him/her a medical examination. As a part of the medical tests we would like to collect a small sample of 5 ml of blood from your child using a sterile syringe. We will rub in a skin anaesthetic cream an hour before we take blood so that there will be no pain. We would also like to test your child's immunity by making some very small pricks in the skin and measuring the response. A small hard area may develop around the pricks which may itch, but this is not harmful.

During this next stage of our research into *Ascaris* in your community we will provide free medical treatment to you and all your children less than 10 years old if they are ill. The treatment centre will be open from Sunday to Thursday between 2 pm and 4 pm at our office on Row 2, Block C in Section 11.

We will leave this form with you overnight so that you can think about it and get advice from your husband. We will return to collect the form tomorrow. If you agree to take part we ask you to sign or give your thumb print. You may withdraw your child from the study at any time you wish.

Thank you for your continued cooperation with our work.

Signature or thumb print of mother:

ASCARIS STUDY - HYGIENE AND ENVIRONMENTAL CONTAMINATION

Part 1. Details to be recorded about household.

Location of household
Ethnic origin of mother
of father
Number of rooms
Number of people sleeping in household
- age
- gender
- relationship to mother of study child

Type of floor - in house (earth or cement)
in compound (earth or cement)

Facilities within household - water source and type
- latrine and type
- separate kitchen
- electricity

Part 2. Questions to be asked of mother of study child

What is your usual source of water for drinking?
What is your usual source of water for washing plates?
What is your usual source of water for washing clothes?

[If latrine in household]
Does your latrine ever overflow when it rains?

[If no latrine in household]
Where do you usually go to defecate?
Where does your child usually go to defecate?
Where is the nearest latrine?

For each child being studied

How often does your child bathe?
Where does he/she bathe?
Do you bathe your child or does he/she wash him/herself?

Does your child was his/her hands after defecation?

Does your child bite his/her finger nails?
Do you cut your child's finger-nails?
If yes - how often?

Does your child suck its fingers?
If yes - how often
Does your child suck objects such as toys or clothing?

Does your child wash his/her hands before eating meals?
If yes, what does he/she use?

Does your child buy snacks from vendors or people on the street?

Where does your child usually play?
Do you restrict the movements of your child?

Have you ever seen your child putting earth in its mouth?
If yes - how long ago?
If within last year, how many times?

Has your child passed any worms within the last month?

Do your babies or children ever defecate inside the house or compound, even accidentally?
If yes - does it happen often?
- how do you remove the faeces?
- where do you throw it away?
- do you wash the spot afterwards?
If yes - how?

Maternal education and knowledge of *boro krimi* = *Ascaris*

Did you attend school?
If yes, what sort of school?
for how many years?

Can you read?
If yes, what language?
Can you write?
If yes, what language?

Do you think that it is normal for children to have *boro krimi*?

Do you think that *boro krimi* does any harm to children?

Do you know how children get *boro krimi*?

Can you tell when your children have *boro krimi*?
If yes, how do you know?

What are the symptoms of *boro krimi*?

Would you treat your child if you thought it had worms?
What sort of treatment would you seek?

[For each child taking part in the study]

Do you think that he/she has *boro krimi* now?
Do you think that he/she usually has many worms, few worms or in between?

Why do you think that some children have lots of *boro krimi* while others have very few?

What other types of *krimi* do you know of?

সম্মতি পত্র

বাড়ী নং _____
শিশুর নাম _____
মায়ের নাম _____

আপনারা জানেন যে, প্রায় দুই বছর ধরে আমরা আপনার শিশুর দৈহিক গড়ন মেপে আসছি। আপনাদের এলাকার ১,০০০ (এক হাজার) জনের এবং আপনাদের শিশুদের পেটের কেঁচো কৃমি আমরা ৩ (তিন) বার সংগ্রহ করে ছিলাম। আমরা আবিস্কার করেছি যে- কিছু লোকদের পেটে অনেক কৃমি বার বারই রয়ে যায় অপর অন্যদের খুব কম। এই কারণে আমরা আরও কিছু গবেষণা করতে চাই যে, কেন কিছু শিশুর পেটে সবসময় অনেক কৃমি রয়ে যাচ্ছে। তার জন্য আপনার শিশুদের গবেষণায় অংশ নিবার জন্য আপনাদের অনুমতি চাই। যাদের অনেক কৃমি আছে তাদের সাথে যাদের অনেক কম তাদের একটা তুলনা করার জন্য কিছু পরীক্ষা করব।

প্রথমতঃ পূর্বের মতই আমরা আপনার শিশুর ও অন্যান্যদের কিছুটা মলের নমুনা সংগ্রহ করে- তা পরীক্ষা করার পর কৃমি পড়ার জন্য একটা ঔষধ খাওয়াব এবং পূর্বের মত আমাদের দেওয়া একটা বালতিতে পর পর দুই দিনের সবমল এবং কৃমিগুলি সংগ্রহ করতে বলব। পূর্বের মতই এবারও আমরা এর জন্য প্রত্যেককে ১৫ (পনর) টাকা করে তাতাদিব। আপনাদের অবসর সময়ে আমরা আপনাদের পরিবার সদস্যদেরকে কিছু প্রশ্ন করব। আমরা আপনার বাসার চতুপার্শে থেকে কিছুটা মাটি ও ধুলাবালির নমুনাও সংগ্রহ করব-তাতে কেঁচো কৃমির ডিম আছে কিনা- তা পরীক্ষা করার জন্য।

ঐ চিকিৎসা করার এক মাস পর আমাদের ডাওগর সাহেব আপনার শিশুদেরকে পরীক্ষা করবেনঃ একটি বিশুদ্ধ সিরিঞ্জ দ্বারা কিছুটা রক্ত (০৫ মিঃ লিঃ এরমত) সংগ্রহ করবেন তবে আপনার শিশু ব্যথা পাবে না- এমন ধরনের একটা এনীম রক্ত নেওয়া ১ ঘন্টা পূর্বে লাগানো হবে। এবং ঐ শিশুর রোগ প্রতিরোধ ক্ষমতা দেখার জন্য তার চামড়াতে খুব ক্ষুদ্র সুঁচ ফুটিয়ে পরীক্ষা করব। তারচার-পাশে- একটু- চুলকাতে পারে এবং খানিকটা শক্ত হতে পারে-তবে এটা ক্ষতি কারক নয়। কেঁচো কৃমির উপর আমাদের এই পরবর্তী গবেষণায় অংশ নিলে আপনাদের এলাকাতে আমরা আবারও বিনামূল্যে চিকিৎসা করব যে খানেই আপনারা অসুস্থ্য হয়ে পড়েন। আমাদের চিকিৎসা কেন্দ্র বৃহস্পতিবার থেকে রবিবার পর্যন্ত প্রত্যেহ বিকালঃ ২টা হইতে ৪টা পর্যন্ত খোলা থাকবে। (চিকিৎসা কেন্দ্রঃ লেন-২, ব্লক- সি, বাসা নং- ১৩ সেকশন-১১, মিরপুর, ঢাকা)।

আমরা আপনাকে এই ফরমটি আজ দিয়ে যাব, যাতে আজ রাতের মধ্যে আপনারা চিন্তা করে স্বামীর পরামর্শ নিবেন। আমরা আগামী কাল এই ফরমটি ফেরৎ নিব। আপনারা যদি রাজী থাকেন তবে নীচে আপনার স্বাক্ষর বা বৃদ্ধা আংগুলির ছাপ দিন।
যখন ইচ্ছা আপনি আপনার শিশুকে এই গবেষণা কার্য্যক্রম হইতে বাদ দিতে পারেন।
আপনাদের, গবেষণা কাজে আপনাদের অবিরত সহায়তার জন্য অনেক ধন্যবাদ।

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স্বাস্থ্য কর্মীর স্বাক্ষর-

_____
মাতার স্বাক্ষর / বৃদ্ধা আংগুলির ছাপ

তারিখ _____