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

1. Source of Population:
   a) Ill subjects (Yes) No
   b) Non-ill subjects Yes No
   c) Minors or persons under guardianship (Yes) No
2. 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 possibly damaging to subject or others Yes (No)
3. 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
4. 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
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 ... an overview ... required ... be submitted with individual ...
   - ✓ Protocol (Required)
   - ✓ Abstract summary (Required)
   - ✓ Statement given or read to ... nature of study, risks, types of questions to be asked, and right to ... participate or withdraw (Required)
   - ✓ Informed consent form for subjects
   - ✓ Informed consent form for ... guardian
   - ✓ Procedure for maintaining ...
   - __ Questionnaire or interview ...

* If the final instrument is not ... prior to review, the following ... should be included in the ...

1. A description of the ... covered in the questionnaire or interview which could be considered sensitive or which would ... invasion of privacy.
2. Examples of the type of questions to be asked i... n ...
3. An indication as to when the questionnaire will be presented to ... for review.

We agree to obtain approval of the Review Board on Use of Human Volunteers ... changes involving the rights and welfare of subjects before making such ...

[signature]

Principal Investigator

Train...

82

Rcd. 21 July 19.

77-008

SECTION I - RESEARCH PROTOCOL

1) Title: F. Buski

2) Principle Investigator: R. Gilman.

3) Starting Date: July 1, 1977

4) Completion Date: September 1, 1968

5) Total Direct Cost: 20350

6) Abstract Summary:

This study will examine the life cycle and environment necessary for Fasciolopsis buski. Patients infected with F. buski will have symptoms recorded, be examined and the number of worms expelled determined. Village epidemiologic studies on growth and infection with F. buski will continue. Using the indirect flourescent antibody test studies on F. buski sero-epidemiology will be performed. Infection of rabbits with metacysts will be performed. Rabbits developing F. buski infection will have pathological studies.

7) Reviews: (Leave blank)

a) Research Involving Human Subjects: _____

b) Research Committee: _____

c) Director: _____

d) BMRC: _____

e) Controller/Administrator: _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objective: The objective of this study is to understand the role of F. buski in growth retardation and clinical symptoms. Also the life cycle of F. buski in Bangladesh will be elucidated.

2. Background: Fasciolopsis buski, the large intestinal fluke was discovered in 1843 by Busk in a Lascar sailor in London. The parasite besides occuring in man is present in pigs. The chief endemic area has been in the Kwangtung and Chekiang Provinces of China. It has also been found in the Indochina, Thailand, Malaya, Indonesia, Formosa and India (Bihar). The fluke inhabits the small intestine usually the duodenum where it attaches itself to the mucosa by a ventral sucker. It may also be found in the stomach or in the large intestine. The life cycle described in man by Barlow in 1925 is as follows:

The egg after a week to two weeks in water hatches into a miracidium. The miracidium swims actively and usually infects a suitable snail host within two hours. There are three types of snails which can be infected two of which (Segmentina and Gyralus) are probably

present in Bangladesh (personal observation). Cercariae develop in the infected snail within a month. These are released from the snail and swim to a nearby water plant at which point they encyst. Metacysts are ingested when the water plants (such as water chestnut or water caltrop) is eaten or the skin peeled by mouth. The adult fluke will then develop within the duodenum or upper jejunum of the human or animal host. Most studies on symptomatology of Fasciolopsis buski were done prior to 1953 and the symptoms described usually are those of abdominal discomfort, nausea, malabsorption type stools and with heavier infection edema and anasarca. This description would also fit that of Kwashiorkor and it is not at all sure whether or not the early description of the symptoms of heavy Fasciolopsis buski infection is not just a description of children who have kwashiorkor. In patients with heavy infection, leukocytosis and eosinophilia are also described. Intestinal changes associated with flukes consist of localized foci of inflammation occuring at the site of attachment in the duodenum and upper small intestine.

Lesions develop involving the capillaries of the intestinal wall and producing hemorrhage or abscesses with infiltration of small round cells and eosinophils. In addition there may be an excess secretion of mucus. [1,2]

In Bangladesh there is a unique situation in which there is a small, well circumscribed endemic zone of Fasciolopsis buski. There appears to be no animal reservoir host as the pig, the usual animal host in other countries, is not present in these areas. This is the only geographical area described in the literature in which the pig is not intimately associated with the Fasciolopsis buski life cycle. Prevalence rates of 40% have been found in one village located east of Dacca.[3] The effect of F.buski infestations on the health of the host is controversial. It would appear from Plautt's studies in Thailand that light infection may have little effect on the nutritional status of Thai children. Plautt found no differences in the nutritional state between two matched groups of children where the only difference was the presence of F.buski infections.[4] He did not report on the intensity of infection. Early studies with heavily infected patients (i.e. above three hundred flukes per patient) have reported symptoms of diarrhea, anasarca, edema, asthenia eventually causing death.[2] These studies are based mainly on single case reports

and controlled observations are not available. It would appear that similar to hookworm and trichuris infestation, Fasciolopsis buski infestation may produce symptoms if a high worm burden is present. We have had children with mild symptoms and over 200 worms evacuated. We have, however, also seen children with over 500 worms who had hypo-proteinemia and edema. No studies on the relationship between worm burden and intestinal protein loss are available. Helminthic prevalence figures are often misleading since symptoms appear to corrrelate more directly with the intensity and duration of worm infection. No study examining the intensity of infection in relationship to nutritional parameters has been performed. In addition, the life cycle of F.buski in Bangladesh has not been described. Knowledge of snail dynamics and plant life present in Bangladesh is at best scanty.

The epidemiology of F.buski is at best in its early stages and would be helped by the development of a serological test. Manning has reported the presence of complement fixation antibody against F.buski in patients infected with this fluke. Seroepidemiological studies in populations infected with Fasciolopsis buski have not been performed previously. The mechanism by which the fluke produced intestinal disease have not been well

defined and pathological studies are extremely limited. Through the establishment of the F.buski life cycle, it may be possible to infect rabbits with F.buski. Rabbits could then be examined for pathological effects produced by this parasite.

3. Rationale: F.buski may become more widespread. This would prove possible if it is a recently introduced infection or dependent, in the absence of a reservoir host, on a dense population. Its cycle and effects on the human host therefore are important from both a preventative as well as a curative viewpoint. In addition F.buski does not have an invasive tissue cycle (lung, skin or liver) but directly attaches to the small intestine. This makes it an interesting model, one which could be used to study the local immune response to parasitic infection and immune expulsion of the parasite.

B. SPECIFIC AIMS

This protocol will be divided into the following areas: 1) life cycle and environmental studies 2) village studies 3) clinical treatment studies 4) development of methods for sero-epidemiology and 5) pathological studies.

Specific Questions

A. Environment and Life Cycle

1. Is the water the same at infected and uninfected site?

2. Are the snails the same and do they also have equal abilities to acquire infection?

3. Are the plants different? Do they have metacysts?

4. Which snails can be infected by F.buski?

Season and change in numbers and percent infection.

B. Village Studies

1. Does heavy buski infection cause retardation of growth?

2. Is infection seasonally acquired?

3. Is there an increase in other protozoal parasites or enteropathogenic bacteria associated with heavy F.buski infection compared to less infected controls.

4. Is Meheran truly a non-infected village?

C. Hospital Studies

1. Relation of worm load to MIF counts.

2. Small intestinal X-ray changes.

3. Is there protein loss?

4. Small bowel pathology.

D. Sero-epidemiology

1. Presence of antibody in patients with F.buski.

2. Correlation with intensity of infections.

3. Antibody specificity.

E. Pathology - Intestinal changes in humans and in rabbits during F.buski infection - variables - intensity of infection and in

rabbits' duration of infection.

C. METHODS OF PROCEDURE

Environment and Life Cycle - This portion of the study is under the direction of Mr H. Rahman and L. Rutherford of the Livestock Research Institute of the Bangladesh Government and Dr R. Gilman and Dr S. Aziz of CRL. Why does the region east of Dacca have a high endemic foci where as preliminary observation has shown a geographically similar areas such as Meheran in Matlab appears to have no Fasciolopsis buski infection? Possible differences in these two areas could be the snail host, the type of plant present or physical changes in the water. Preliminary observations appear to show little difference in snail type or the majority of water plants known to be significant in F.buski transmission. The plant shapla-root and stem is eaten uncooked in both areas. Although it is not known for Bangladesh it would appear reasonable to consider that the majority of infection occurs in late monsoon or immediately post-monsoon.

Two areas will be sampled: one will be an area known to be endemic for F.buski infection, other will be an area in which prior preliminary studies in young children have revealed no infection with F.buski. The non-endemic region will serve as a control in which to compare the dynamics of infected versus non-infected snails. Snail density, identity and percent infected

with cercariae will be determined four times a year with sampling from at least ten different tanks in each area. The pre-monsoon sampling at the endemic village has just been completed and has shown an infection rate in Planorbidae snails at less than 5%. Half the study has been completed at Meheran where snails of Gyralus species (probable snail identification) have also been found. No infection was found in any of 208 snails of the Planorbidae type examined from Meheran. Historically, it was found that water caltrop, water chestnut and water lilies were common in the endemic area during the monsoon or post-monsoon season. Early results show that water lilies are present and eaten raw in Meheran. Preliminary pre-monsoon water sampling in both study areas showed little difference in coliform, ph or turbidity determinations. Tanks which have been previously sampled will again be sampled in the post-monsoon (September) season for the following parameters:

1) Snail Density

   a. This will be performed by a man hour count of snails. Each tank will have two counts of 15 minutes each. The number of snails counted in this period of time will be identified and then studied for cercarial infection by crushing. The type of cercariae will be described and then fixed for further identification.

The infective form is a metacyst found on plants in the areas where water caltrop, chestnut or lilies are found. Sampling will be performed in a 3 yard square area over four quadrants of the tank in August - October and December. All plants will be identified and each species identified from a particular tank will then be stored in the refrigerator for future metacyst identification. Water from tanks in Meheran and an endemic village will be sampled every 3 months and the following determination made, coliform counts, biological dissolved oxygen, turbidity, ph, and if possible calcium and chlorides.

These studies will continue every three months until October of 1978 in an attempt to establish both the seasonality, infection rate and plant species necessary to produce an endemic region of F.buski. In addition, 2 monthly sampling of two tanks in the endemic village in which only planorbidae type snails will be studied for cercarial infection will be undertaken. In addition, a control tank at Meheran will also be sampled at the same time. At present, there is a less than 5% infection rate of planorbidae snails; assuming that 20% of planorbidae snails during monsoon will be infective with cercaviae. We will need to examine 100 snails per 2 months to establish a difference in the seasonal prevalence of snails infected with F.buski.

Life cycle studies: At present it is not known which snails are susceptible to F.buski infection in Bangladesh. Previous studies have been relatively limited in other parts of the world. Studies on infecting groups of clean snails of six varieties will be performed. Snails classified as Planorbidae (Gyralus and Segmentina) will be given first preference. In addition, endoplanorbis, bithynia and lymnaea will also be infected. Approximately 50 clean snails will be exposed to a hundred miracidium of F.buski. Miracidium will be obtained from F.buski eggs obtained from adult flukes. The eggs will be incubated under distilled water for two or three weeks. We are assuming a snail infection rate of 50%. Snails after 14 days and weekly thereafter will be tested for cercarial release by exposure to a strong light for 3 hours. The cercariae if present will be allowed to encyst on the side of the glass. Metacysts will then be collected and fed in varying dosages to rabbits. Rabbits have been shown to be another animal host beside the pig in which the life cycle can precede to completion. It should be possible to find which species of snail are able to become infected by and complete the snail stage of F.buski. The understanding of the life cycle of F.buski, its snail host, its environment and type of water plant in the environment may provide us with a more complete understanding of the means necessary to eliminate this infection from Bangladesh.

E. FACILITIES REQUIRED

The Laboratory facilities required for this work will be provided by the Livestock Research Institute except for studies on metacyst infection of rabbits and plant identification.

In terms of CRL facilities - one refrigerator will be needed for storage of plants for a period of approximately 3 - 6 months. One half of a Revco will be utilised.

Animal resources - 16 rabbits per documented infected species of snail will be utilised. Eight rabbits will be given, if possible 150 metacysts and sacrificed at 60 days and 90 days. Major logistical support will be eight two day trips to Meheran for each period of surveillance. Weekly car trips to and from the endemic village for the next twelve months.

F. COLLABORATIVE ARRANGEMENTS

Collaboration is with Mr H. Rahman, M.Sc and Elizabeth Rutherford, B.Sc. Details of the budget will be provided in the final budget of the whole project.

PART II

Field Studies

Preliminary studies on 400 children in any endemic area have been performed. Data is now being collated from these studies. Preliminary studies show that one egg per methiolate iodine formalin (MIF) 2 mg smear is equivalent to about 5 - 10 F.buski flukes. At present time analysing data broken into single age groups only we have not obtained a significant correlation coefficient between buski counts and Hct or serum specific gravity. We have now found a village area which is much more convenient to use, and after an initial survey, which will include census and mapping, stool will be collected for parasites, using Formal ether, MIF and PVA. Height, weight, capillary blood for total protein and buski antibody, midarm circumference and skin fold will be determined. Once MIF counts have been read we will divide the group by egg count intensity and perform longitudinal studies.

As longitudinal studies are more sensitive we hope to show a 30% difference in growth rates of children with heavy infection compared to those without heavy buski infection. Assuming that only 50% of the children with heavy buski infection will have a 10% growth rate compared to 80% of the children without heavy buski we will need 40 children in each of the following three groups.

1) Children in each age group with an infection rate of over five eggs per 2 mg smear of F.buski (Group 1) will be studied. Each child will be compared to children of the same age in the

village who have either an infection rate of 1 thru 5 eggs per 2mg smear (Group 2) or no infection on 2 mg smear (Group 3). It is expected that there will be approximately 40-60 children who have heavy infection. The studies performed will be a 3 monthly determination of height, weight, midarm circumference and skin-fold thickness. Stool examination will be performed using formal ether concentration method, methiolate iodine formalin for ova counting and polyvinyl alcohol for protozoa identification. Rectal swab, will be directly streaked onto MacConkey #3 and XLD agar. The presence of enteropathogenic bacteria will be determined by routine measures. All groups will have treatment with antepar at the end of each bleeding session. Vitamins will be supplied at each measuring session. Specific anti-buski treatment will be withheld for 6-9 months. At the end of 6-9 months if no nutritional differences are found half the children will be treated with F.buski medicine hexylresorcinal and the other half will be given antepar. The second clinical study will involve children from the ages group 2-5. One hundred and fifty children who have had two samples prior to August 30th negative for the presence of F.buski by formal ether examination will be included in the study. One stool on these children will be examined by formal ether examination every two months to determine an age specific rate of F.buski acquisition over a seasonal period of time. This assumes that the majority

of changes in stool positivity for F.buski will occur in the first quarter. This information should provide data correlating human acquisition of infection with plant and snail seasonality. A prevalence study of helminths utilizing 8 year olds at Meheran will be performed. Finger tip blood and stool will be collected and processes as previously described. As this group has been found to have the highest prevalence rates in studies from Bangladesh; if no F.buski is found we should be able to say F.buski infection is probably non-existent at Meheran. We already have data on the 0-4 years age groups, which shows no F.buski in a sample of over 100 children.

Finally studies examining the border zone of the endemic region are planned. These studies will provide us two control villages. One village with a prevalence rate of 20% and the other a village with no buski infection but a high degree of similarity to positive villages. A separate protocol will be provided for these studies. At present we do not know the correlation of serology with stool prevalence of F.buski.

The facilities for the field studies are the following. Initially a vehicle will be needed daily for 2 months. Every three months thereafter, daily vehicle transport to the endemic

village will be required (for a period of 2-3 weeks). Male and female epidemiologists, field assistants trained in antropometric techniques will be required, the laboratory technician who can draw blood and one recorder will be required. The field team will be under the direction of an unpaid volunteer Josephine Harrison. Laboratory space - approximately 30 formal ether specimens can be examined by one technician per day. Approximately 12 MIF specimens can be counted per day by one technician. Approximately 10 PVA specimens can be stained and examined by one technician per day. PVA specimens will only be examined if the presence of cysts or trophozoites are revealed in the MIF or formal ether specimen. The initial survey will take 2 months to complete with two full time technicians. The longitudinal study will take two technicians approximately one month of each three months full time for identification purposes. The acquisition rate study will only be performed with formal ether specimens. Thus this study will only require two weeks of two full time technicians' time. Laboratory space required for these examinations - it is hoped that the Public Health Services Laboratory will be available for the year August to August. Hospital resources will be described under clinical treatment program. Physical analysis for the longitudinal study will

consist of a student test for the differences per three-month period in height and weight changes between the three groups. In addition Chi Square analysis or Fisher's exact test will be used to evaluate differences in number of enteropathogens found in each group. This study assumes that there will be less than a 10% change in the number of patients who change infectivity status. These patients will be dropped from analysis. Analysis of seasonality will be performed using Chi Square test since only prevalence is being established. No animal resources will be required. The only major item of equipment is a international scale (65 kgs); one length stick and one pair of skin calipers. This study will be done in collaboration with Dr Muttalib of the Bangladesh Health Association.

Group III Clinical Treatment Studies - Patient recruited from either the village for patient who have F.buski present on stool examination, as an outpatient will be admitted to the CRL study ward. The only requirement for admission will be the presence of buski eggs on stool examination. The patient after treatment of any primary condition causing diarrhea such as: Shigellosis, Amebiasis or Cholera will after 2-3 consecutive days with soft stools have Methiolate Iodine Formalin (MIF) ova counts performed. Xylose and in a few cases pulmonary breath tests will be performed. Patient will then be fasted and either chlortetraethylene capsules or hexylresorcinol capsules will be given. A purge with magnesium sulphate will follow in two hours. All stools passed will be sieved by having water run over fine netting and the flukes and hookworms expelled identified and counted. All patients will be hospitalized for at least three days after initial therapy and if complete clearance has not occurred will be given a second dose at the end of four days. Assuming one week inpatient hospital stay and a total of 25 patients so treated will provide the cost. CBC differential count, rectal swab will be done. Children over the age of 8 will have an upper G.I. with fluroscopic visualization performed. We do not plan on performing more than 10 of these X-ray procedures. Married girls will not be included. Duodenal tubes maybe passed and barium injected directly into the small bowel through the tube. Also, in cases with heavy infestation, a small bowel biopsy will be taken, fixed in formal-saline, glutaraldehyde and another portion frozen. They will

be described pathologically and immunoglobulin cells identified. Each patient will have an age-matched patient with giardia selected as a control. These patients will have the same initial admission studies performed and also have in some cases a small bowel X-ray. No small bowel biopsies will be performed in these children.

Development of serological method for sero-epidemiology of F.buski. Developmental studies using fluke antigens and flourescent antibody tests will be attempted to be developed at the Cholera Research Laboratory. The only previous study of serology on this parasite have used an extracted antigen and a complement antibody test. Fluke extract has been used as an antigen for immunization of rabbits. Sera from these rabbits give us a titer of 2500 against frozen fluke sections. Early studies appear to show that infected humans also produce antibody to fluke antigen. It is felt that flourescent antibody test using cryostat sections of F.buski fluke may provide a good method of establishing infection in the community. In addition, this method may allow us to examine the relationship between the intensity of infection with F.buski and either the presence of antibody of its titer. Sera to be used will be from patients at the Cholera Research Laboratory and patients from an endemic village where capillary blood has been drawn. This sera are at present available at the CRL. Sera will also need to be taken from the non-endemic area of Meheran to establish that cross reaction with other parasites such as ascaris trichuris and hookworm does not produce an antibody rise to this parasite.

Method - Flukes obtained after tetrachlorethylene treatment will be frozen and put into tissue tek and trimmed to a convenient size. Five micron sections will be fixed for 15 minutes in acetone and then put into a -70 freezer for storage. Sera from patients will be serially titered from 1-10 to 1-1240 using doubling dilutions. Slides will be incubated with the appropriate dilution. The slide will be washed in phosphate buffer for a period of 10 minutes and a total of three washes. After washing, slides will then be incubated with fluorescence conjugated anti human gammaglobulin reagent. Controls will be run using sera taken from expatriates newly arrived from the United States, who have never travelled in areas where the infection is endemic and sera from patients living in Meheran, a non-emdemic region. One thousand samples of sera will be run for F.buski antibody. The sera will come from an endemic village, Meheran and hospitalized patients. This will take one full time technician approximately three months of work. Analysis will depend on the shape of the serological curve since if a uniform distribution is not achieved geometrical mean titers will not be usable. Assuming a uniform distribution of titers will be compared with age, prevalence data, intensity of infection and hospitalized patients the duration of antibody titer in an endemic village. Tests of significance will be performed using Chi Square test or student T-test.

Pathological Studies - These studies will depend on the successful establishment of the life cycle of F.buski in Bangladesh. They will

be performed in association with Dr Moin Islam. The number of rabbits needed for a grid-like study will be 72. There will be six rabbits in each experimental group. Rabbits will be uninfected, infected with 15 metacercaria, 150 metacercaria or 500 metacercaria. Rabbits will be examined 15 days, 45 days and 90 days after infection. Daily weights will be taken. Description of stool will be provided daily. Any rabbit who dies will be examined by post-mortem. Any rabbit that appears in an agonal state will be sacrificed and post-mortem performed. The intestine of rabbit will be fixed in formalin after the intestine is opened and pinned prior to fixation. Prior to fixation photography may be taken. The number of flukes present will be counted and identified. Tissue samples taken from the point of attachment of fluke on the mucosa will be taken for flutaraldehyde fixation. Another section will be snap frozen in alcohol and dry ice or if available liquid nitrogen in isopentane for immunoglobulin cell determination. Sections will be stained with H&E, Trichrome and alcian blue. "All animals will have weekly sera drawn for antibody determination." This project will hopefully give us data on the mechanism by which F.buski infects the host and the resultant tissue reaction to this infection. It is hoped that this will provide insight into the pathological mechanism by which this parasite produces disease. This study will be a preliminary study.

Facilities required will be one month of one histology technician's

time for blocking, cutting and staining. In addition one month of Dr Moin Islam's time for interpretation of the intestine. Glutaraldehyde - Fixed specimens will be shipped back to Baltimore for further analysis.

ABSTRACT SUMMARY

Clinical Study -

Children with Fasciolopsis buski will be admitted to hospital. A barium swallow will be given to children over 10 years and fluroscopy at 30 minutes and 60 minutes performed. Fluroscopy time will be limited to 3 minutes total and gonads will be shielded. A duodenal tube may be placed and dye administered through this tube.

Small bowel biopsies will be taken in children over 5 years of age with F.buski infection. Prior to biopsy, a blood donor will be identified and then kept on hand for 48 hours. Twenty-four hours after biopsy or at the onset of any abdominal symptoms an erect chest X-ray for the presence of free air will be taken. A surgeon will be present in Dacca who, in case of an emergency, we can refer the patient to.

All children will have two days of stool examinations and routine treatment of pathogens. On the third day a dose of Piperazine will be given and on the fourth day a dose of either hexylrescorcinol or chlortetraethylene will be given, both effective and recognized agents for treatment of F.buski. Purge with Magnesium sulphur will follow. The number of worms present will be counted. If not cleared of eggs, children will have a second dose of medicine given. Children will be asked to return one month after therapy.

Children will have blood on admission and on discharge.

HOSPITAL CONSENT FORM

I understand that my child has a worm called F.buski. I also understand that he will receive medicine for this illness. The medicine may make his nauseous. After receiving the medicine he will receive another medicine to wash out the worms. He will also have to save all his stools so that the number of worms can be counted. He will also have small amounts of blood (5-10cc) collected for examination from the arm on two or three occasions. I understand that if my child has poor digesting power he may swallow a tube. He may then have a small piece of tissue snipped from his intestine for examination. This examination may in a few cases produce bleeding and in one in 20,000 can even make a hole in the intestine which would require a major operation for repair and could result in death. I understand that my child will swallow chalk and then have an X-ray of his stomach and intestine. I realize that this X-ray will expose him to more radiation than he would normally receive but is below the maximal permissable dose for one year. He may require 2 treatments to get all the worms out. Also I understand that if I refuse I will not be penalized in any way from receiving the usual medical care. I also realize that I can withdraw from this study at any time and will still receive routine therapy.

Signature of Guardian _____ Date _____

ACQUISITION STUDY

I realise that my child will take part in a study of worms in which stool samples will be collected 4 times a year.

I realise that I am free to refuse participation in this study and that refusal will not prejudice treatment at CRL hospital in any way. I also realise that I can withdraw my child from this study at any time and in no way be penalized.

Signature _____ Date _____

STATEMENT IN FIELD

I am willing to have my child take part in a study in which his height, weight and arm size will be determined. I also will give 2 stools 4 times a year for examination. Fingertip blood will be taken once each year.

A rectal swab will be taken 4 times a year to examine whether my child has bacteria which could cause diarrhea.

I am free to refuse this study for my child and this will in no way be penalized for doing so. Also, I may withdraw my child's participation in the study at any time without jeopardizing therapy for F.buski or any other disease at Cholera Hospital.

_____ _____

Signature Date

Field Study

Children with heavy buski infection will be compared to children with no or slight buski infection, in terms of growth, other parasites and enteropathogens. Rectal swabs will be taken with the oldest child being 10 years of age. Each year one finger stick blood will be obtained for measuring specific gravity, hematocrit and antibody to F.buski levels. Nutritional parameters (height, weight, mid arm circumference and skin fold) will be determined 4 times per year. Children will be treated with antepar and vitamins. After 6-9 months children with heavy infection will be divided into 2 groups if no difference ingrowth rate has been found and half the group treated with hexylrecorcinol.

A separate group of children in whom no evidence of F.buski has been found will have only stools examined 4 times a year for evidence of acquisition of F.buski.

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SECTION III - BUDGET

A. DETAILED BUDGET

PERSONNEL SERVICES

Name	Position	% of Effort	Annual Salary	Project Requirements TAKA	Dollars
1. R. Gilman	JHU	20%	$ 33,000.00	102,300.00	6,600.00
2. Bob Black		10%	$ 46,000.00	71,300.00	4,600.00
3. E. Retherford	JHU VSO British	90%	Tk. 18,000.00	16,200.00	1,045.16
4. H. Rahman	JHU Div of Livestock	70%			
5. J. Harrison	Volunteer	90%	No salary		
6. Kris Faegradus	Volunteer	100%	Tk. 18,000.00	18,000	1,161.29
7. Dr. Seaton		2%	$ 18,907.00	5,861.17	378.18
8. Dr. S. Aziz		10%	Tk. 10,010.00	1,001.00	64.58
9. Dr. W. Spira		2%	$ 32,750.00	10,152.50	655.00
10. Study Doctors:					
a) Dr. Rabbani		20%	Tk. 20,084.00	5,417.00	350.00
b) Dr. Asma		10%	Tk. 27,084.00	5,417.00	350.00
11. Henry Ghose	Clerk	30%	Tk. 6,312.00	1,893.60	122.17
12. Parasite Tech	JHU	50%	Tk. 10,000.00	5,000.00	322.58
13. Parasite Tech	JHU	70%	Tk. 7,200.00	5,040.00	325.16
14. Parasite Tech	JHU	50%	Tk. 3,600.00	1,800.00	116.13
15. Histology Tech Mrs. Pashi		20%	Tk. 21,309.60	4,262.00	275.00
16. Serologist Joe Gomez		35%	Tk. 15,927.60	5,574.66	359.66
17. Immunochemist		20%	Tk. 15,000.00	3,000.00	194.00
18. Card Puncher		2%	Tk. 2,604.00	52.08	3.36
19. Secretary		15%	Tk. 21,808.00	3,272.00	211.00
20. Urban Epidemiologist (Maksud)		20%	Tk. 2,174.00	435.00	28.05
21. a) 1 Field Supervisor (2 months) August to Sept. 15 3 weeks every 3 months afterwards			Tk. 25,443.60	7,421.00	479.00
b) 1 Field Assistant (2 months) August to Sept. 15 3 weeks every 3 months afterwards.			Tk. 8,205.60	2,893.00	186.64
c) 1 Field Assistant (3 weeks) every 3 months thereafter			Tk. 8,205.60	4,615.65	297.78
d) 2 Local persons			Tk. 9,600.00	9,600.00	619.35
e) Overtime - Joe Gomez (4 hrs./6 days)				1,413.60	91.20
			SUB TOTAL:	291,921.26	18,835.29

SUPPLIES AND MATERIALS

Items	Amount Required Taka	Dollar
1. Rectal Swab Cultures 150x4	10,350	700
2. Natelson Tubes 10 packets	2,170	140
3. Plastic Vials, 1000	233	15
4. Histologic Supplies:	775	50
Absolute Alcohol, 10 lit.	775	50
Alcohol 95%, 25 liters	674	43.50
Xylene, 25 liters	333.75	21.50
Paraffin	310	20
Stains	2,325	150
Immune Sera	3,875	250
Knives	2,325	150
Buffer Salts	775	50
Rabbits, 125	6,250	404
Boots -Field, 52	310	20
Hewlett-Packard Calculator	9,300	600
Stencils	310	20
Stationery	155	10
Pens	310	20
Syringes 100x10 cc. 4 box	203	14
Flourescent Bulbs, 2	2,170	140
1 Dram Serum Bottles, 1000	1,860	120
Stool Containers, 1000	465	30
Miscellaneous	6,984	450.58
Sub Total:	53,042	3,422.

7. One trip Calcutta - 4 days per diem = $ 200.00

8. PRINTING & REPRODUCTION

Xerox	=	$ 150
Others	=	$ 50
Publication costs	=	$ 500
Sub Total:		$ 700

9. CONTRACTUAL SERVICES

M. Islam (6 hours a week)
Pathologist

10. CONSTRUCTION, MAINTENANCE = $ 200

3. EQUIPMENT

Item	
1. Cryostate	$ 3810
2. Tissue Processor	$ 1850
3. Microtome	$ 1254
4. Leitz Flourescent Microscope	$ 4000
5. Revco - ½	$ 1200
6. Refrigerator 1+½	$ 300
7. Paraffin Dispenser	$ 385
8. AO Microscope	$ 1242
9. Dissecting Microscope	
10. Photo-Microscope Unit - Needed	
11. Books - Snails - Malek	
Trematodes - Dawes	
12. Air Conditioner - 2	$ 600
13. Power Points 1-2	Tk. 50
14. Scale - 65 kg.	$ 800
15. Skin Calipers	$ 100

4. PATIENT HOSPITALIZATION

Inpatient - Days = 150 x 135	= Tk. 20,250	= $ 1,306.45
CBC = 15 x 50	= Tk. 750	= $ 48.39
Stool= 15x150	= Tk. 2,250	= $ 145.16
Sub-Total:	Tk. 23,250	$ 1,500

5. Out patient follow up = 10% Urban Epidemiologist (Maksud) Tk. 217.40 $ 14.03

Sub Total: Tk. 217.40 $ 14.03

6. CRL TRANSPORT

3,000 Miles	= Tk. 4200	= $ 270.96
10 days Boat - 40 hours running time	= Tk. 3954	= $ 255.10
Sub Total:	Tk. 8154	$ 526

FACILITIES

OFFICE SPACE:

a) Laboratory space	400 sq.feet for Parasitology Lab. Section - 15 months.	
b) Laboratory space	300 sq.feet for Histology Lab. - 15 months	
c) Hospital Resources	CRL - Inpatient 175 - 5 months	Tk. 23,625.00 $1524
d) Animal Resources	Rabbits - 125 for 2 months	Tk. 6,250.00 $ 403
e) Vehicles	2,000 miles over a year period	Tk. 4,800.00 $ 181
f) Major items	Histology Lab.	
g) Special Diets	Food for Snails - Sterilized letruce	
h) Office Space	R. Gilman, J. Harrison, Dr Rabbani and Dr Asthma	

Collaborative arrangement - An informal collaborative arrangement between Dr. H.Rahman, E. Ruther Ford and myself has been ongoing. We have been supplying transport, use of a disecting microscope and miscellaneous supplies.

BUDGET SUMMARY

Category	Year 1	Year 2
1. Personnel	13,562	
2. Supplies	3,422	
3. Equipment		
4. Hospitalization	1,500	
5. Outpatients	140	
6. CRL Transport	$ 526	
7. Travel Persons	$ 200	
8. Transportation of Things		
9. Rent/Communication		
10. Printing & Reproduction	$700	
11. Contractual Service		
12. Construction	$200	
	= $ 20,350	

PROCEDURES FOR MAINTAINING CONFIDENTIALITY

Patients admitted to the study will be given a study number; records will be kept according to study number and all data will be kept in a locked file in the investigator's locked office. Following completion of the study, all identifying information will be cut off from the data sheet and the clinical information only will be kept at the Cholera Research Laboratory in a locked data storage office. Results of the study will be published in a medical journal and no identifying information will be included in the report of this study.

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BUSKI STUDY SHEET

Name: _____ Wt: _____

Age: _____ Ht: _____

Sex: _____ No. _____ Dt _____

Date:

	Admn. 1	2	3	4	5	6	Discharge 7
Frequency							
Abd Pain							
Blood							
Mucus							
Quality							
Distension							
Liver							
Spleen							
Nausea/ Vomiting							
Rectal Swab	A			B			C
F.Buski egg in stool							
Rectal Swab							
Anarexia Incoordinating							

Tetrachlorethylene

	Total Fluke	
TCE given on	In stool	In vomiting
TCE given on		
TCE given on		

Egg Count _____

Total Flukes Passed _____

	Hct
	Sp.Gr.
	TWBC
	EOS

F. BUSKI STUDY

Name_____ Age_____ Sex_____

Hosp #_____ Weight_____ Admission Date_____

Chief Complaints: 1) _____ Duration_____

2)_____ Duration_____

3)_____ Duration_____

Flukes in stool_____ Duration_____ In Vomitus_____

Stool character: Watery ☐ Dysentery ☐ Loose ☐ Soft ☐ Formed ☐

Number of stool per day_____ Duration.

Diarrhea more than 4 loose stool/day Yes ☐ No ☐ Duration_____

Vomiting : Yes ☐ No ☐ How long_____

Urticaria : Yes ☐ No ☐ How long_____

Cough : Yes ☐ No ☐ How Long_____

Fever : Yes ☐ No ☐ How long_____

Wt. loss : Yes ☐ No ☐ How long_____

Resides how far from : Ponds/Tanks/Canals/River _____

Main source of water : Tubewell/Tanks/Deep Well/Canal/River. Dry when_____

Does the pt eat water plants_____(1)Shapla (2)Shingara(3)Shalook(4)Other

Water plants taken : Raw ☐ Cooked ☐

When taken last_____ Villages lived in before_____

Other members with Buski_____ Duration_____

Prior Medicine taken_____ Did flukes pass?_____

Other associated illness_____