REVIEW BOARD ON THE USE OF HUMAN VOLUNTEERS Date September 6
CRL 80

Principal Investigator R. Gilman Trainee investigator(if any) _____

Application No 77-024 Supporting Agency(if Non-CRL) _____

Title of study F. Bush - Epidemiology — Project status:
( ) New Study
(X) Continuation with change
( ) No change (do not fill out rest of form)

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

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 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 Board for review.

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

R. Gilman
Principal Investigator

Trainee

Please return 2 copies of entire protocol to Chairman, Review Board on Use of Human Subjects.

Attachment 1a

INFORMATION TO INCLUDE IN ABSTRACT SUMMARY

The Board will not consider any application which does not include an abstra t sum
The abstract should summarize the purpose of the study, the methods and procedure
be used, by addressing each of the following items. If an item is not applicable
please note accordingly:

1. Describe the requirements for a subject population and explain the rationale
for using in this population special groups such as children, or groups whose
ability to give voluntary informed consent may be in question.

2. Describe and assess any potential risks - physical, psychological, social, l
or other - and assess the likelihood and seriousness of such risks. If meth
of research create potential risks, describe other methods, if any, that were
considered and why they will not be used.

3. Describe procedures for protecting against or minimizing potential risks and
assessment of their likely effectiveness.

4. Include a description of the methods for safeguarding confidentiality or pro
ing anonymity.

5. When there are potential risks to the subject, or the privacy of the individ
may be involved, the investigator is required to obtain a signed informed co
statement from the subject. For minors, informed consent must be obtained f
the authorized legal guardian or parent of the subject. Describe consent p
cedures to be followed including how and where informed consent will be ob

   (a) If signed consent will not be obtained, explain why this requirement
   should be waived and provide an alternative procedure.

   (b) If information is to be withheld from a subject, justify this course
   of action.

6. If study involves an interview, describe where and in what context the inter
will take place. State approximate length of time required for the intervie

7. Assess the potential benefits to be gained by the individual subject as well
the benefits which may accrue to society in general as a result of the plann
work. Indicate how the benefits outweigh the risks.

8. State if the activity requires the use of records (hospital, medical, birth,
death or other), organs, tissues, body fluids, the fetus or the abortus.

The statement to the subject should include information specified in items 2,3,4
7, as well as indicating the approximate time required for participation in the a

Received 9/9/77

77-024

SECTION I - RESEARCH PROTOCOL

1) Title: Epidemiology - F.buski

2) Principal Investigator: Robert H. Gilman, M.D.

3) Starting Date: September 1, 1977

4) Completion Date: September 1, 1978

5) Total Direct Cost: $16,977

6) Abstract Summary:

A fasciolopsis buski endemic village will have a census performed. Stool samples from children (0-12) and all women in the village will be examined by formal ether and Methiolate-Iodine-Formalin technique. Ova counts will be determined. Nutritional anthropometrics will be measured and the hematocrit and total plasma protein determined. Antibody titers to endamebae histolytica and Fasciolopsis buski will be determined. Villagers will be followed quarterly to establish seasonality of acquisition and to follow growth rates.

7) Reviews:

a) Research Involving Human Subjects: _____

b) Research Committee: _____

c) Director: _____

d) BMRC: _____

e) Controller/Administrator: _____

SECTION II - RESEARCH PLAN

A. INTRODUCTION

1. Objective: To determine the effect of F.buski on nutritional parameters in a population based study.

2. Background: Fasciolopsis buski, the large intestinal fluke was discovered in 1843 by Busk in a Lascar sailor in London. The parasite, besides occurring 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 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:[1,2,3]

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,[1] two of which (Hippeutis and Gyralus) are definitely present in Bangladesh (personal observation). Gymnocephalus cercariae develop in the 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 and 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 after a period of a month or more.

Metacysts appear to attach non-specifically to any plant which they are

near. Edible plants therefore are suitable vectors only because they are eaten.[3]

F.buski in large numbers has been associated with anasarca and edema. This is probably kwashiorkor since these syndromes were described prior to 1930. In Dacca, 1-2% of children hospitalized at the Nutrition Unit for malnutrition have relatively high F.buski worm burdens and associated kwashiorkor. (Personal observation)

Population based studies on the nutritional effect of F.buski are lacking. The only study performed to date has been a study by Plautt.[5] He matched a child infected with F.buski with a similar child who was not infected with F.buski. Nutritional and biochemical parameters were measured. No major differences were found between the two groups of children. This study is inadequate for the following reasons. Unfortunately, the intensity of infection in this study is never specified by either ova counts or worm burdens. They do refer to patients treated at hospital but not involved in the study. These patients had low worm burdens with a mean of 13 worms per patient. This should be contrasted with patients in China where worm burdens over 1,000 are recorded.[7] The mean worm burden of patients seen in Dacca at ICDDR,B is also over 100 per patient. Bradley has pointed out that to examine parasitic effects by population based methods very high prevalence of a parasite are required. The curve of worm burden in a high prevalence area is a log-normal curve.[6] Thus, intense infection will only be present in 10-20% of an infected population. This, of course, disregards special occupational hazards which may allow intense infection to occur by clustering. It becomes apparent that unless the presence of a single parasite itself causes significant and frequent

pathology, or is self replicatory i.e. hydatid cysts, ill effects will only be correlated with the intensity of the parasitic infection.

In most intensity dependent parasitic diseases, pathological effects are most easily demonstrated by a marker. The marker for hookworm is anemia, in S.hematobium, hematuria, in tricurisis, rectal prolapse and chronic diarrhea and in S.mansoni, heptomegaly. Parasites in which markers are absent are more difficult to study. Ascarsis, probably world-wide the most effective parasite of man, has not been shown to produce ill effects in population based studies.[7] In micro-studies, nitrogen balance is improved after roundwork treatment.[8] Recently, a study from India has purported to show that growth is affected. This study used supplemental feeding and a longitudinal study design to demonstrate these changes.[9]

F.buski has no pathological marker. Edema and anasarca are relatively rare even in heavily infected children (personal observation). We suspect that protein loss through the intestine is the major pathological feature of F.buski infection. Thus, in population based studies, a major criteria of morbidity would be the level of serum albumin or its close correlate, the total plasma protein. One would also expect that if chronic protein losing enteropathy and/or malabsorption is the basis for disease, retardation of growth would occur. Preliminary studies in Dacca show that one egg per methiolate iodine formalin (MIF) 2 mg smear is equivalent to about 5-10 F.buski flukes.

In F.buski, seasonal transmission has been assumed to occur during monsoon

and late monsoon time.[2] During the winter and spring months, the snails hibernate so that few are present for infection. Also, the only time acquatic plants are eaten is during the monsoon and late monsoon season. Thus, we have an infection probably occurring only in one season of the year. This should be possible to demonstrate. The rate of parasite loss is also unknown but is probably less than 15% per year. It would therefore be unusual for children, once characterized as negative, to change their status after October or November. Also, heavy infections would tend to remain heavy throughout the year.

Only one report of sero-epidemiology has been published using a complement fixation assay.[10] In this assay, low levels of cross-over in other helminths were found but patients with F.buski infection had higher titers.

We have found that frozen sections of fluke can be used to provide a relatively specific but somewhat insensitive test for the presence of F.buski. We screen serum at a titer of 1:20. Heavily infected patients have had titers of up to 1:160. Patients without F.buski have had weak reactions at a 1:10 dilution but so far have been negative at a titer of 1:20. We are now using smears of adult flukes which are then fixed in acetone. This allows us to do more sera on the same slide and decreases processing time. It is expected that in the next two months a standardized indirect fluorescent antibody test for F.buski antibody will be available to use as a sero-epidemiological tool.

Rationale: Assuming F.buski produces effects on small bowel function,

these effects should be measurable in ultimate terms of decreased growth.

A study comparing intense infection in a villlage with marginal nutrition and both a high prevalence of F.buski and aggregates with high intensity of worm infections should be suitable for studying population based effects of a parasite. Longitudinal studies have been included in an effort to monitor growth prospectively.

A population of children with a high prevalence of F.buski infection will have ova counts of F.buski determined. These counts will be compared with their nutritional status using age independent indicators. The effect of F.buski on growth rates will also be determined by stratifying patients with different levels of infection into three groups (a) no infection, (b) light infection, (c) severe infection and then comparing growth rates of these three groups on a quarterly basis.

Indirect fluorescent antibody titers to F.buski will be determined. By using serum from a control village where no F.buski has been found we will determine the specificity of the antibody test. By titering the sera of an endemic village and comparing the titer with ova counts, we will be able to determine the sensitivity of the test.

Children below 4 years of age have low rates of F.buski, thus this group can be monitored for a change in status. This group will be studied quarterly to determine the season of acquisition. In addition, a group of infected children will be studied to determine spontaneous loss of infection.

B. SPECIFIC AIMS

1) To determine the effect of F.buski infection on nutritional parameters.

2) To determine if heavy F.buski infection is associated with infection with other protozoal or enteric pathogens.

3) To determine the correlation of a positive indirect fluorescent antibody test with the presence of F.buski. Also, to determine the effect of age and intensity of infection on titers.

4) To determine if F.buski infection is acquired seasonally.

C. METHODS OF PROCEDURE

N.P., a village of approximately 500 children below the age of 12 years, will be sampled. An initial census will be performed and the village will be mapped. Children below the age of 12 and their mothers will consititute the sample population.

Stool will be collected for parasites, using Formal ether, MIF and PVA. Height, weight, capillary blood for total protein and buski antibody and E.histolytica 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. These children 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 or 2mg 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[13] and polyvinyl alcohol for protozoa identification. Rectal swab, will be directly streaked onto MacConkey SS 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.

Treatment - If no significant difference in groups is shown by 6 months, half the children with heavy infection will be treated for F.buski and the other half will not be treated. These groups will then be followed quarterly for the next 6 months.

Any child found to be severely malnourished (below 65% height for weight, International Standards) will be referred for hospitalization. In addition, children with dysentery will be given specific treatment. Children or mothers with hematocrits below 5% will be given iron and folic acid therapy.

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-endemic 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 four 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.

Indirect fluorescent antibody test will be performed using smears made of F.buski flukes. Smears are dried, fixed in acetone for 5 minutes and again dried. Sera will be screened at a 1:16 dilution. This dilution will be used since it is the recognized starting dilution for E.histolytica antibody test. Slides will be incubated with sera for 30 minutes, washed and then incubated for 30 minutes with a 1:20 dilution of anti-human globulin labelled with fluorescein.

In addition, hemaglutination titers to Endamebae histolytica will be performed by standard methods. This will provide data on invasive E.h and will be correlated with F.buski ova counts.[13,14]

All data will be tabulated and collected directly on computer-coded sheets. This data will then be punched on cards. The computer will then be utilized in tabulating, and if possible, in performing some statistical tests such as a simple correlation matrix.

SIGNIFICANCE

To use a population based survey to establish whether F.buski infestation may contribute to host malnutrition.

FACILITIES REQUIRED

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 3-4 weeks). Male and female epidemiologists, field assistants trained in anthropometric 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. Office space will be one desk for J. Harrison, either at JHU or Epidemiology Division, and one desk for Maksud at Epidemiology. 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 8 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 September to September. 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 assumed 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 an international scale (65 kgs), one length stick and one pair of skin calipers.

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ANNEX III

PERSONNEL SERVICES

Name	Position	% of Effort	Annual Salary	Project Requirements Taka	Project Requirements Dollars
1. R. Gilman	Principal Investigator	10%	$ 33,000		3,300
2. B. Black	Co-investigator	8%	$ 46,000		3,680
3. J. Harrison	Volunteer	95%	-		-
4. M. Khan		5%	Tk.60,210	3,010	
5. I. Huq		5%	Tk.66,305	3,315	
6. A. Ahmed		5%	Tk.64,257	3,213	
7. Field Assistants (4)					
(Maksud)		90%	Tk.11,436	10,292	
(Hassma)		40%	Tk.10,241	4,096	
(Other)		40%	Tk.10,241	4,096	
(Jaimal Abedin)		one month	Tk.14,492	1,208	
Matlab		only off			
Experienced bleeder		station			
8. Two local persons				9,600	
9. Parasite Technician*	JHU	75%	Tk.10,000	7,500	
10. Parasite Technician*	JHU	50%	Tk. 6,000	3,000	
11. Parasite Technician*	JHU	50%	Tk. 8,000	4,000	
12. Serologist		40%	Tk.21,808	8,723	
13. Mrs Pashi					
(Histology Technician)		20%	Tk.21,309	4,262	
14. Serologist (2)					
Joe Gomez		5%	Tk.15,927	796	
Huda		5%	Tk.15,927	796	
15. Secretary		20%	Tk.21,808	4,362	
16. Statistician		20%	Tk.21,967	4,393	
17. Bacteriology Technician		10%	Tk.13,255	1,326	
18. Joe Gomez or					
Clinical Pathologist					
48 hours overtime				200	
			Total	68,198	6,980

Note - Joe Gomez will receive 2 hours overtime for 4 weeks. Samples from the village arrive at 5:00 P.M. Serum specific gravity has to be run immediately. Overnight refrigeration allows precipitation and false values to result. Freezing precipitates fibrinogen so this also produces false values.

		Taka	Dollars
2.	Supplies		
	Vials Caraway tubes, Stool cups, serum bottles, stencils Fluorescent reagents - slides		3,000
3.	Equipment		200
4.	Patient Hospitalization (20 days)	Tk.3,000	
5.	Outpatient Care and Stool ME (40 visits)	Tk.2,500	
	Items 4 and 5, patients referred from the village for therapy.		
6.	CRL Transport	Tk.2,000	
7.	Overseas and Local Travel		200
8.	Transportation		600
9.	Rent, Communication and Utilities		50
10.	Printing and Reproduction		
	Xerox		200
	Other		50
	Publication Costs		500
11.	Contractual Services		
	Mapping		100
12.	Construction		50

: 1 : ABSTRACT SUMMARY

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 of 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 in growth 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.

Children with evidence of dysentery or severe diarrhea will be returned to the CRL hospital for examination. Malnourished children (65% of the international standard) will be referred for therapy to the Nutrition Research Unit Hospital. Villagers with hematocrits below 25% will be treated with iron and folic acid.

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.

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 penalised.

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 penalize me. Also, I may withdraw my child's participation in the study at any time without jeopardizing therapy for F.buski or any othe disease at Cholera Hospital.

_____ _____

Signature Date