

CRL

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

Application No 77-022 Supporting Agency(if Non-CRL) 88

Title of study F. buski - Life Cycle Project status:
 New Study
 Continuation with change
 No change (do not fill out rest of form)
Nonhuman Research -

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
- 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
- Does the study involve:
 - a) Use of records (hospital, medical, death, birth or other) Yes No
 - b) Use of fetal tissue or abortus Yes No
 - c) Use of organs or body fluids Yes No
- Are subjects clearly informed about:
 - a) Nature and purposes of study Yes No
 - b) Procedures to be followed including alternatives used Yes No
 - c) Physical risks Yes No
 - d) Sensitive questions Yes No
 - e) Benefits to be derived Yes No
 - f) Right to refuse to participate or to withdraw from study Yes No
 - g) Confidential handling of data Yes No

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.

Principal Investigator R. Gilman Trainee _____

INFORMATION TO INCLUDE IN ABSTRACT SUMMARY

The Board will not consider any application which does not include an abstract summary. The abstract should summarize the purpose of the study, the methods and procedures to 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, legal or other - and assess the likelihood and seriousness of such risks. If methods 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 an assessment of their likely effectiveness.
4. Include a description of the methods for safeguarding confidentiality or protecting anonymity.
5. When there are potential risks to the subject, or the privacy of the individual may be involved, the investigator is required to obtain a signed informed consent statement from the subject. For minors, informed consent must be obtained from the authorized legal guardian or parent of the subject. Describe consent procedures to be followed including how and where informed consent will be obtained.
 - (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 interview will take place. State approximate length of time required for the interview.
7. Assess the potential benefits to be gained by the individual subject as well as the benefits which may accrue to society in general as a result of the planned 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 and 7, as well as indicating the approximate time required for participation in the activity.

13, 14, 15

susceptible to infection in only one area. Therefore, for each geographical area the life cycle in all susceptible vectors needs to be established. This is done by 3 methods 1) examining wild snails for cercariae, 2) infecting snails in the laboratory to show susceptibility and 3) infecting animals with metacysts from these infected snails.

The latter step is necessary since a gymnocephalus cercariae is a morphological type only. Specific pathogen identification is not possible. In Bangladesh 3 pathogens are endemic in which gymnocephalus type of cercariae are present. These are *Fasciolopsis buski*, *Fasciola hepatica* and *Fasciola gigantica*. All 3 of these trematodes have a gymnocephalus cercariae which cannot be differentiated by present methods. Fortunately, the snail types appear not to overlap.

Meadows infected with *Fasciola hepatica* can be surveyed by feeding grass to guinea pigs. Similar techniques have not been tried as a survey method for *F. buski* infection. The rabbit is a susceptible laboratory host animal for *F. buski* infection.

In the past few months the following data has been obtained by the investigators involved in this project.

1. Snails of hippautis species have been found in both the endemic and non-endemic areas. *Hippeutis umbilicalis* is a known snail vector for *Fasciolopsis buski*. In addition, 3 *hippeutis umbilicalis* snails from the endemic region have been found to be infected with a gymnocephalus type cercariae.
2. Water plants such as shapla (water lily) are present in endemic

and non-endemic regions like Meheran. Historically, it was found that shapla, although cooked by older people, is eaten raw by children, usually after peeling the skin of the stem by the teeth or biting into old flower pods.

3. The pig, although present, is relatively rare and is mainly limited to Harijan or Christian villages. It has not been found near either of two villages which are hyperendemic for *Fasciolopsis buski*. Thus a man to man cycle is assumed.

3. Rationale: The understanding of the life cycle is essential to trematode control. Since there is a high degree of host specificity in trematodes for their snail intermediates. Susceptibility to infection varies not only with different species but also with the same species in different geographical areas.

Therefore, the following 3 methods will be used to show proof of involvement. Specimens collected in the endemic and non-endemic region will be examined for *gymnocephalus cercariae*. The base line infection with non-human *gymnocephalus cercariae* will be provided by the non-endemic region. Further establishment of the susceptible species will be by infection of snails in the laboratory derived from both endemic and non-endemic regions. If the snails from the non-endemic region can be infected this will provide considerable evidence that conditions are suitable for the introduction of *F. buski* infections.

Assessment of the intensity of infection in one area may be possible by feeding plants contaminated with metacysts to rabbits. This would provide a simple survey method if found sensitive. Plants from a non-endemic region will act as a negative control.

B. SPECIFIC AIMS

- 1) Establishment of the snail intermediates of *F. buski* in Bangladesh.
- 2) Suitability of areas for introduction of *F. buski*.
- 3) Seasonal changes in snail density and infectivity with *F. buski*.
- 4) Metacyst identification and use of animal feeding studies to survey an endemic *F. buski* focus.

C. METHODS OF PROCEDURE

Two areas will be sampled. One an area known to be endemic for *F. buski* infection and the other will be an area in which prior studies in young children have revealed no infection with *F. buski*. A non-endemic region will serve as a control in which to compare the dynamics of planorbidae snails only. These snails will be compared for infectivity, numbers and species present. In a non-endemic region three tanks will be studied. One tank is a bathing tank with relatively slight vegetation around the edges. Another tank is a latrine tank which floods out to the main canal and is heavily filled with water hyacinth and into which several latrines directly send their effluent. The last tank is a derelict, water hyacinth filled tank which is used primarily for clothes washing. In the endemic area, three tanks previously shown to have planorbidae snails present will be sampled every two months. These three tanks consist of 1) a bathing tank, 2) a latrine tank which during monsoon time consolidates with several other tanks and 3) a portion of flooded paddy field where shapla is found growing. Specifically, the stems of the shapla plant will be checked for the presence of snails. This is because it has been noted that encystment takes place relatively close to the area in which the snail is present when releasing its cercariae.

Coliform counts will be determined on these tanks every two months. There will be an attempt to related fecal contamination with infectivity rate and snail population changes. Snail density 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. Since it appears that cercarial encystation will occur on essentially any form of plant surface, only those plants in which either a) snails are abundant such as water hyacinth or edible by man will be tested. Testing will be performed by two methods. Samples from study tanks will be put into plastic with sufficient moisture to prevent dessication. They then will be studied under dissecting microscope and structures thought to be metacysts examined and given to rabbits or pigs. b) a more indirect method is based on studies performed with *F.hepatica* in guinea pigs. Here, samples of shapla, shingara and water hyacinth will be fed to a susceptible host. In this case most likely rabbit. Animals will subsequently be examined for the presence of trematode infection 90 days following infection. The lowest infective dose which can be detected by this method will be established by using plants on which a known number of metacysts have been deposited in the laboratory. These studies will continue every two months until October 1978.

Logistics - In the pre-monsoon sampling there was less than a 5% infection rate of planorbidae snails. Assuming that 20% of planorbidae snails during monsoon will be infected with cercariae we will need to examine 100 snails per 2 months to establish a difference in the seasonal pre-

valence of snails infected with *Fasciolopsis buski*.

Life Cycle Studies: At present, establishment of the life cycles and snails susceptible to *F. buski* infection in Bangladesh is still unknown. We have succeeded in establishing colonies of 2 types of planorbidae snails in the aquarium. Clean snails derived from eggs are now available from one of these. In addition, clean snails of *Lymnea rubingnosa* are also available for infection. Eggs for miracidium production will be obtained from flukes which are immediately dissected to isolate large numbers of infective eggs. Flukes will come from patients treated with an appropriate anti-*buski* agent or passed spontaneously. Eggs will be incubated in distilled water for 4 weeks. At the time they are seen to be mature they will be placed in beakers with 10 to 20 snails. Approximately a 10:1 ratio of miracidium of *F. buski* to each snail species will be maintained. Miracidium infection will be observed through a dissecting microscope. 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 exist on the side of the glass or on a clean underwater grass stem or water hyacinth leaf. Metacysts will then be collected and fed in varying dosages to the rabbits.

1. SIGNIFICANCE

This will provide data essential for future survey work and control programs.

2. FACILITIES REQUIRED

Animal resources - 30 rabbits will be utilized to establish metacyst

Received 9/9/77
77-022

SECTION I - RESEARCH PROTOCOL

- 1) Title: Life-Cycle of F.buski
- 2) Principal Investigator: Robert H. Gilman, M.D.
- 3) Starting Date: September 1977
- 4) Completion Date: October 1978
- 5) Total Direct Cost: \$5,458
- 6) Abstract Summary: (250 words or less)

F.buski is endemic in Bangladesh. Life cycles' studies are necessary to delineate the intermediate snail host vector. Susceptibility of snail hosts to infection with F.buski miracidium from both endemic and non-endemic regions will establish area susceptibility.

Seasonal snail dynamics and natural infection with cercariae of F.buski will be determined. Metacysts will be used to infect rabbits to establish the dose necessary to produce infection. Plants given by weight will be fed to rabbits to survey a given area for metacysts.

- 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: Whether conditions are suitable for *F. buski* in non-endemic regions of Bangladesh.
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^{1,2} 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 (*Hipppeutis* and *Gyrulus*) are ^{definitely} present in Bangladesh (personal observation). *Gymnocephalus cercariae* develop in the snail within a month.^{3,4} 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.²

Metacysts appear to attach non-specifically to any plant which they are near. Edible plants therefore are suitable vectors only because

infection of plants. In addition, 16 rabbits per documented infected species of snails will be utilized for metacyst identification. Dosage of metacyst necessary for infection will require 36 rabbits.

Major logistical support will be 8-10 two day trips to Meheran for periodic surveillance and 8-10 car trips to and from the endemic village site.

Travel - one 4 day trip to Calcutta will be made to review literature on F. buski by E. Rutherford.

Water Studies - fecal counts will be made by B. Spira at 2 monthly intervals from the study tanks.

F. COLLABORATIVE ARRANGEMENTS

Collaboration is with Mr H. Rahman, M.Sc. and Elizabeth Rutherford, B.Sc.

BIBLIOGRAPHY

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3. Nakagawa, K. The development of *Fasciolopsis buski* (Lankester).
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4. Lo, Chin Tsong. 1967 Life history of the snail, *Segmentina hemisphaenla* (Benson) and its experimental infection with *Fasciolopsis buski* infection in Thailand. Tr. Roy. Soc. Trop. Med. 63:470-478
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8. Ahmed, S. and Islam, S. *Fasciolopsis* in Narayanganj. 117:9, 1973
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Oncomelania Formosana to Infection with Human Strains of S. Japmicun.
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14. Ross, J.G. & Hagan, J.O. A Biological Technique to Assess Numbers of Fasciola hepatica metacercariae on Pastures. J. of Hel. 40:375-378, 1966

SECTION III - BUDGET

1. PERSONNEL SERVICES

Name	Position	% of Effort	Annual Salary	Project Requirements	
				Taka	Dollars
1. R. Gilman	Principal Investigator	5%	\$ 33,000		1,650
2. H. Rutherford	Volunteer VSO	90%	--		
3. H. Rahman	Division of Livestock	20%	Tk. 18,000	3,600	
4. Dr S. Aziz		5%	Tk. 10,010	500	
5. Dr B. Spira		5%	\$ 32,750		1,638
6. Dr Mahmood		5%	Tk. 20,000	1,000	
7. Dr Kaddas		5%	Tk. 20,000	1,000	
8. One field assistant		10%	Tk. 6,000	600	
9. One animal house assistant		30%	Tk. 8,000	2,400	
10. One bacteriology research technician		10%	Tk. 14,000	1,400	
			Total	<u>10,500</u>	<u>3,288</u>

2. SUPPLIES AND MATERIALS

Aquariums	5,000
Rabbits	5,000
Snail supplies	500
Stencils	500
Misc.	500
Sub-Total:	<u>11,500</u>

3. EQUIPMENT

1,500

4. PATIENT HOSPITALIZATION

N/A

5.	<u>OUTPATIENT</u>	N/A	
6.	<u>CRL TRANSPORT</u>		1,000
7.	<u>OVERSEAS TRAVEL</u>		
	4 days Calcutta		2,000
8.	<u>TRANSPORTATION</u>		800
9.	<u>RENT</u>		800
10.	<u>PRINTING AND REPRODUCTION</u>		
	Xerox		3,000
	Other		800
			<hr/>
		Sub-Total:	3,800
			<hr/>
11.	<u>CONTRACTUAL SERVICES</u>		500
12.	<u>CONSTRUCTION</u>		150

ABSTRACT SUMMARY

F.buski is endemic in Bangladesh. Life cycles' studies are necessary to delineate the intermediate snail host vector. Susceptibility of snail hosts to infection with F.buski miracidium from both endemic and non-endemic regions will establish area susceptibility.

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