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Date 23/06/87

...ICDDR,B

...l Investigator ANDREAS HAHN Trainee Investigator (if any) 21.

...ation No. 87-018 Supporting Agency (if Non-ICDDR,B)

...f Study DETECTING THE METABOLITES OF ASCARIS LUMBRICOIDES IN URINE

Project status:
(✓) New Study PILOT
( ) 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).

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 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
(h) 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 N/A 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.

(PTO)

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

Principal Investigator

J... 5 1987

Trainee

87-018
23.06.87

SECTION 1 - RESEARCH PROPOSAL

1.	TITLE	: Detecting the metabolites of *Ascaris lumbricoides* in urine
2.	PRINCIPAL INVESTIGATOR	: Andrew Hall
3.	STARTING DATE	: When the money is available
4.	DATE OF COMPLETION	: 1 year after start
5.	TOTAL COST	: $2 350
6.	SCIENTIFIC DIVISION HEAD	: Dr. David Sack

This proposal has been approved by the Laboratory Sciences and Epidemiology Division.

SIGNATURE OF SCIENTIFIC PROGRAMME HEAD: David G. Sack DATE: 25.5.87

7. ABSTRACT

Ascaris lumbricoides is one of the most common parasites of man in the world today. If morbidity due to *Ascaris* is to be controlled then infections must be diagnosed and treated. *Ascaris* is usually diagnosed by finding eggs in the faeces of infected people, but the microscopical examination of faeces is time consuming, requires equipment and is therefore expensive and not really suitable for use in mass treatment campaigns. A simple diagnostic technique which can be used in the field is required.

Ascaris is reported to be unique in that it produces two short chain fatty acids as end products of its carbohydrate metabolism: 2-methyl valerate and 2-methyl butyrate (Bryant, 1982). It is proposed to examine the urine of people infected with *Ascaris lumbricoides* by gas liquid chromatography to detect short chain fatty acids, and if unique constituents are present when compared with the urine of uninfected controls, to relate the concentration of these substances to the worm burden. This will be the first stage in investigating the chemistry of the urine of people infected with *Ascaris* with the aim of providing a simple test of urine to make a roughly quantitative diagnosis.

8. REVISIONS

1. Ethical review committee.

2. Research review committee.

3. Director.

SECTION II - PLAN OF RESEARCH

A. INTRODUCTION

Ascaris lumbricoides is probably the most common intestinal worm of man in the world today. It has been estimated that over a quarter of the world's population may be infected, and in some countries such as Bangladesh, over 80% of children may have Ascaris. (Martin et al, 1983).

Adult Ascaris live in the small intestine and fertilised females produce about 200,000 eggs a day. The eggs can be found by examining faeces under a microscope and the diagnosis made is specific. However, infections with immature females or males alone may not be detected for the simple reason that no eggs are produced. In addition, light worm burdens of fertilised females may be missed if only a small amount of faeces is examined under the microscope: examining more faeces will increase the likelihood of making a diagnosis but tends to increase the time spent on detecting an infection and so increases the cost. All these factors have to be taken into consideration when planning mass treatment campaigns to reduce the prevalence of infection. What are the costs and efficacy of selective treatment based on diagnosis rather than treating the whole community?

Another question raised about selective rather than mass treatment revolves around identifying "wormy people". Because the distribution of worms in a community tends to be clumped, with up to 70% of worms aggregated into 20% of hosts (May, 1985), would treatment aimed at these wormy people be more effective at reducing both the average intensity of infections and morbidity than treating all infections? Mathematical models tend to indicate that it would (Anderson & May, 1982). However, once again the problems of diagnosis recur: a quick, reliable and cheap diagnosis in the field with a quantitative estimate of the worm burden is not possible. Faecal samples have to be examined under a microscope by a trained technician, while an estimate of the concentration of eggs requires a weighed sample to be analysed, perhaps after the eggs have first been concentrated by techniques of centrifugation. All this costs time and money and requires expensive equipment.

Ascaris is a large parasite with a significant biomass: female worms can grow to be up to 35 cms long and 7 g in weight, and a worm burden of over 200 g is not uncommon. Like many other parasites, Ascaris meets its energy needs primarily by glycolysis. Unlike other parasites however, two of the end-products of its energy metabolism are unique: it ferments carbohydrates to produce 2-methyl-butyrate and 2-methyl-valerate (Bryant, 1982). It is likely that these compounds are excreted by the worms into the intestine of the host. There they may be absorbed and then metabolised by the host, or are perhaps excreted unchanged in the host's urine. Some research on volatile fatty acids in the urine of Ascaris infected pigs has found unusual constituents and suggests that their concentration is related to the worm burden (Soprunova et al, 1973). If the presence and approximate concentration of unmetabolised short chain fatty acids in the urine of people could be detected simply and easily in the field then it could provide a new means of diagnosing and quantifying burdens of Ascaris lumbricoides.

The research proposed here has two stages. First, to examine by gas liquid chromatography urine samples from people infected with Ascaris lumbricoides both before and after deworming. Secondly, if unique constituents

are found which are present in concentrations proportional to the worm burden, to try and develop a reagent which when added to urine will indicate the presence of Ascaris lumbricoides in the intestine, ideally in approximate proportion to the worm burden. Only research aimed to examine the first stage is proposed here: if it is successful then the second stage may be undertaken.

B. AIMS

To examine the volatile organic compounds found in the urine of people infected with Ascaris lumbricoides using gas liquid chromatography and to relate any compounds detected to the size if the worm burden of the host.

C. METHODS

A number of people infected with Ascaris lumbricoides will be identified by a quantitative faecal examination using an ether sedimentation technique (Hall, 1981). The mothers of young children in the Nutrition Unit of the Dhaka Hospital will be approached as these women spend from one to two weeks in the hospital looking after their children. The Physician in charge of the unit has agreed to this. It is highly likely that many of these women will be infected with Ascaris. When they enter the unit with their children they will be asked to provide a faecal sample. In total ten infected women with a range of egg counts would be asked to take part in the study; other women found to be infected but who are not asked to take part in this study will be treated.

Each infected but otherwise healthy woman who agrees to take part in the study will be asked to provide fresh urine and faecal specimens for 3 - 5 consecutive days. Each woman will then be given a single dose of pyrantel pamoate (11 mg/kg body weight of pyrantel base) and will be asked to collect her bowel movements for the following 48 h in order to collect the worms. Another dose of pyrantel will then be given to expel any remaining worms and all stools will again be collected. Urine specimens will be collected for the another 3 to 5 days following treatment and a final stool sample will be collected and examined for the eggs of Ascaris to check that all the worms have been expelled.

Ten female volunteers found to be uninfected with Ascaris will be identified from members of staff of the ICDDR,B. If their stools are found not to contain the eggs of Ascaris they will be asked to provide urine specimens over 5 consecutive days of good health: they will act as uninfected controls.

All faecal samples will be fixed in weighed bottles containing PVA/Schaudinns fluid, reweighed and then examined after ether sedimentation. This will give an estimate of the concentration of eggs in faeces in eggs/g.

All urine specimens will be stored on ice after collection. Volatile organic compounds will be extracted from the urine in a suitable organic solvent and then separated by gas liquid chromatography using a microcapillary column. The actual laboratory procedures will depend on initial experiences with the chromatography of urine.

Samples of urine from uninfected people will be spiked with known concentrations of methyl butyrate (Sigma M 0516) and methyl valerate (Sigma M

1982) in order to provide standard solutions and urine for comparison with samples from infected people. Organic acids produced by intestinal bacteria (butyric, isovaleric, acetic, succinic) which may also be present in urine will be identified using pure preparations added to the urine of uninfected people and in pure form in a suitable solvent.

The concentration of volatile organic compounds in the urine of women infected with Ascaris will be related to the concentration of eggs in faeces, to the number of worms expelled after treatment and to the biomass of the worm burden. The concentration of volatile organic compounds in the urine of women infected with Ascaris will be compared both before and after treatment and with a number of uninfected controls

D. SIGNIFICANCE.

The detection of unique chemicals in the urine of people infected with Ascaris lumbricoides in proportion to the actual worm load could be the first step in establishing a new and simple diagnostic technique for this very common infection.

E. FACILITIES AND SERVICES REQUIRED

1. Use of gas liquid chromatograph in Biochemistry laboratory.

G. REFERENCES

Anderson RM, May RM (1982). Population dynamics of human helminth infections: control by chemotherapy. Nature 297: 557 - 563.

Bryant C. (1982). Biochemistry. In: Modern Parasitology pp 84 - 115, ed. F E G

Hall A. (1981). Quantitative variability of nematode egg counts in faeces: a study among rural kenyans. Trans.R.Soc.Trop.Med.Hyg 75. 682 - 687.

Martin J, Keymer A, Isherwood RJ, Wainwright SM (1983). The prevalence and intensity of Ascaris lumbricoides infections in Moslem children from northern Bangladesh. Trans.R.Soc.Trop.Med.Hyg 77: 702 - 706.

May RM (1985). Ecology and population biology. In: Tropical and Geographical Medicine. pp 152 -166, eds KS Warren and AAF Mahmoud. New York: McGraw-Hill.

Soprunova NJ, Soprunov FF, Lur'e AA. (1973). Nachweis von Helminthen-Metaboliten im Harn des Wirtes als ein neuer diagnostischer Test fur Helminthiasen. Angew. Parasit. 14: 11 - 17.

ABSTRACT SUMM

Ascaris lumbricoides is very common parasite in Bangladesh. In this study a number of otherwise healthy but infected people would be identified and urine samples would be collected from them over a period of about 12 days both before and after treatment. Their treatment will be supervised by a physician. They would be asked to collect all their faeces in a bucket for 48 hours after each treatment. There are no risk to the infected people, only benefits.

SECTION III - PERSONNEL

Principal investigator: Andrew Hill

MSc Student:

DETAILED BUDGET

	US $
Personnel	
Contribution to student's fares etc @ Tk 500/month	200
Supplies and Materials	
Drugs	100
Glassware	250
Stationery and office supplies	200
Chemicals and media	500
Non-stock supplies (hydrogen and microcapillary column)	1000
Printing and publication	100
TOTAL	2350

International Centre for Diarrhoeal Disease Research, Bangladesh

CONSENT FORM

Detecting the metabolites of Ascaris lumbricoides in the urine.

Many people in Bangladesh are infected with the intestinal worm Ascaris, called "kechucrimi" in Bangla. We are doing a study to test the urine of people infected with Ascaris to see if we can tell if they are infected from substances in the urine. This could form the basis of a new diagnostic test. For this work we need to collect stools and urine from a number of people infected with Ascaris and, for comparison, from a number of uninfected people.

If we find that you are uninfected we would like to double check by collecting another stool sample, and then we would like some samples of urine over the next week or so. This will tell us about the urine of uninfected people.

If we find that you are infected with Ascaris then this is what we would like to do:

1. We want you to give us samples of your urine throughout the day for 3 to 5 consecutive days. We will provide the bottles.

2. After this we will give you medicine to treat the worms. We would like you then to collect all your stools for the following 48 hours after treatment so that we can recover and count all the worms that pass out in the faeces.

3. Then we will collect another faecal sample to check that all the worms have been expelled. If they haven't we will give you another dose of medicine.

4. After we have given you the medicine we would like more urine samples for another 3 to 5 days after treatment.

In this way we can test the urine of infected people both before and after treatment and see what differences there are in the urine.

(To be read to women taking part whose children are the the Nutrition rehabilitation unit.)

This study should not interfere with the care you give your child and there is no obligation to take part just because your child is being treated here. Also, you will be free to to drop out of this study at any time particularly if your child is well enough to go home, and we will provide treatment for your worms. But while you are in the hospital we hope that you will agree to take part in our work.

If you agree to take part in this work and to provide us with faecal samples and urine, then please sign below or mark the paper with your finger print.

Thank you.

Signature or fingerprint
of subject: _____

Signature of Investigator: _____

Date: _____

[illegible] গবেষণা কেন্দ্র

সম্মতি পত্র

Detecting the metabolites of Ascariasis lumbricoides in the Urin

(প্রস্রাবে কেঁচোকৃমি জনিত মেটাবলাইটস্ নির্নয়)

বাংলা দেশের বহুলোক কেঁচোকৃমি দ্বারা আক্রান্ত হয়। আমরা একটি গবেষনা চালাচ্ছি যাতে যাদের পেটে কেঁচোকৃমি আছে তাদের পেশাব পরীক্ষা করে পেশাবে কেঁচো কৃমির- জনিত উৎপন্ন কোন বিশেষ কিছু আছে কি না তা বলা যাবে। এটা হতে পারে রোগ নির্নয়ের একটি নূতন পদ্ধতি। এই জন্যে দরকার হবে যাদের পেটে কেঁচো কৃমি আছে তাদের পেশাব ও পায়খানা। এর সাথে তুলনা করার জন্য দরকার হবে যাদের পেটে কৃমি নেই তাদের পেশাব ও পায়খানা।

এতে যদি আমরা দেখি যে, আপনার পেটে কৃমি নেই তবে তা আরও সঠিকভাবে দেখার জন্য আর একবার আপনার পায়খানা পরীক্ষা করবো এবং সবের সপ্তাহে দিয়ে পরবর্তীতে আপনার পেশাবও আর একবার পরীক্ষা করা হবে। এতে আমরা সঠিকভাবে বলতে পারবো যে আপনার পেটে কৃমি আছে কিংবা নেই।

যদি আমরা দেখি যে আপনার পেটে কৃমি আছে তাহলে আমরা নিচের নির্ধারিত বিষয়গুলি করবো:

(১) আমরা আপনার কাছ থেকে পর পর ৩ অথবা ৫ দিন পেশাব রাখবো এর জন্য আমরা আপনাকে বোতল দেবো।

(২) এর পর কৃমির চিকিৎসার জন্য আপনাকে ওষুধ দেবো। ওষুধ খাওয়ার পর আমরা আপনার কাছ থেকে

আপনার স্বাক্ষর

পরবর্তী ৪৮ ঘন্টার জন্য সমস্ত পায়খানা সংগ্রহ করবো যাতে এই সময়ের মধ্যে আপনার পায়খানার সাথে যত কৃমি বের হয় তা যেন আমরা গুনতে পারি।

③ তারপর আর একবার পায়খানা পরীক্ষা করে দেখা হবে যে আপনার পেটের সব কৃমি বের হয়েছে কিনা। যদি না হয় তবে আপনাকে আবারও ওষুধ খেতে দেয়া হবে।

④ আপনাকে এই ওষুধ দেবার পর আপনার কাছ থেকে পর পর ৩ অথবা ৫ দিন পেশাব সংগ্রহ করবো।

এইভাবে আমরা যাদের পেটে কৃমি আছে তাদের কৃমি-চিকিৎসার আগে এবং পরে পেশাবের পার্থক্য পরীক্ষা করে দেখতে পারবো।

(NRU তে যে সমস্ত মায়েরা তাদের বাচ্চা নিয়ে ভর্তি আছে তাদের পরে শোনানো হবে।)

এই গবেষনা চলাকালীন আপনি আপনার শিশুর সব রকম যত্নই নিতে পারবেন, তাতে কোন অসুবিধা হবে না। তাছাড়া আপনি ইচ্ছা করলে যে কোন সময় নিজেকে এ গবেষনা থেকে প্রত্যাহার করে নিতে পারবেন, বিশেষ করে আপনার শিশুর যদি কোন ভালো হয়ে ঘরে যাবার উপযোগী হয়। এতে আপনার কিংবা আপনার শিশুর চিকিৎসার কোন অসুবিধা হবে না, আপনাকে কৃমির চিকিৎসা দিয়ে দেয়া হবে। কিন্তু আপনি যখন হাসপাতালে আছেন তখন আমরা আশা করবো যে আপনি আমাদের গবেষনায় অংশ গ্রহন করবেন।

যদি আপনি এ গবেষনায় রাজী থাকেন তবে নিচে স্বাক্ষর কিংবা বৃদ্ধাঙ্গুলীর ছাপ দিন।

গবেষকের স্বাক্ষর

স্বাক্ষর / বৃদ্ধাঙ্গুলীর ছাপ

তারিখ