



INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH
Mail : ICDDR,B, GPO Box 128, Dhaka-1001, Bangladesh
Phone : 871751-60, Telex : 675612 ICDD BJ
Fax : 880-2-883116, 886050, 871568, 871686 Cable : Cholera Dhaka

MEMORANDUM

CS D
2000

23 August 2000

To : Dr. Wasif Ali Khan
Clinical Sciences Division

From: David A. Sack, M.D.
Chairman, Research Review Committee (RRC)

Sub : Approval of protocol # 2000-022

Thank you for submitting a modified copy of your research protocol # 2000-022 entitled "Molecular epidemiology of cryptosporidiosis" incorporating the observations of the RRC made in its meeting held on 21st August 2000. The protocol is hereby approved upon your appropriate addressing of the observations made by the RRC.

Thanking you and wishing you success in running the study.

cc: Associate Director
Clinical Sciences Division



CENTRE FOR HEALTH AND POPULATION RESEARCH

Mail : ICDDR,B, GPO Box 128, Dhaka-1000, Bangladesh

Phone : 8811751-60, Telex : 642486 ICDD BJ

Fax : 880-2-8823116, 8826050, 8811568, 8811686, Cable : Cholera Dhaka

Memorandum

To: Prof. Mahmudur Rahman
Chairman
Ethical Review Committee

Date: October 3rd 2000

Through: Prof. George Fuchs
Associate Director & Head
Clinical Sciences Division

From: Dr. Wasif Ali Khan
Principal Investigator

Reference: Memo dated September 13th 2000

Subject: Research Protocol entitled: "Molecular Epidemiology of Cryptosporidiosis".

I am pleased to enclose herewith our response to the comments raised by the Ethical Review Committee of the abovementioned proposal, for kind consideration.

(a) The study subjects ranges between 15 days to 60 months. Thus the parents or guardians will sign the Consent Forms. Accordingly on the face sheet item # 5A is changed to 'NO' and item # 5B is kept as 'YES'.

(b) Prof. George Fuchs has signed in the appropriate box and I have signed as the Principal Investigator of the protocol (page 3).

Thanks.

(FACE SHEET)

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator: Wasif Ali Khan

Trainee Investigator (if any): _____

Application No. 2000-022

Supporting Agency (if Non-ICDDR,B) US NIH

Title of Study: Molecular Epidemiology of Cryptosporidiosis

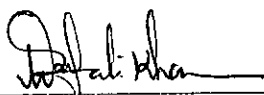
Project Status: _____

 New Study Continuation with change No change (do not fill out rest of the form)

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

1. Source of Population:
- | | | |
|---|---|--|
| (a) Ill subjects | <input checked="" type="checkbox"/> Yes | <input type="checkbox"/> No |
| (b) Non-ill subjects | <input type="checkbox"/> Yes | <input checked="" type="checkbox"/> No |
| (c) Minor or persons under guardianship | <input checked="" type="checkbox"/> Yes | <input type="checkbox"/> No |
2. Does the Study Involve:
- | | | |
|---|---|--|
| (a) Physical risk to the subjects | <input type="checkbox"/> Yes | <input checked="" type="checkbox"/> No |
| (b) Social risk | <input type="checkbox"/> Yes | <input checked="" type="checkbox"/> No |
| (c) Psychological risks to subjects | <input type="checkbox"/> Yes | <input checked="" type="checkbox"/> No |
| (d) Discomfort to subjects | <input checked="" type="checkbox"/> Yes | <input type="checkbox"/> No |
| (e) Invasion of privacy | <input type="checkbox"/> Yes | <input checked="" type="checkbox"/> No |
| (f) Disclosure of information damaging to subject or others | <input type="checkbox"/> Yes | <input checked="" type="checkbox"/> No |
3. Does the Study Involve:
- | | | |
|--|------------------------------|--|
| (a) Use of records (hospital, medical, death or other) | <input type="checkbox"/> Yes | <input checked="" type="checkbox"/> No |
| (b) Use of fetal tissue or abortus | <input type="checkbox"/> Yes | <input checked="" type="checkbox"/> No |
| (c) Use of organs or body fluids | <input type="checkbox"/> Yes | <input checked="" type="checkbox"/> No |
4. Are Subjects Clearly Informed About:
- | | | |
|--|---|--|
| (a) Nature and purposes of the study | <input checked="" type="checkbox"/> Yes | <input type="checkbox"/> No |
| (b) Procedures to be followed including alternatives used | <input checked="" type="checkbox"/> Yes | <input type="checkbox"/> No |
| (c) Physical risk | <input checked="" type="checkbox"/> Yes | <input type="checkbox"/> No |
| (d) Sensitive questions | <input type="checkbox"/> Yes | <input checked="" type="checkbox"/> No |
| (e) Benefits to be derived | <input checked="" type="checkbox"/> Yes | <input type="checkbox"/> No |
| (f) Right to refuse to participate or to withdraw from study | <input checked="" type="checkbox"/> Yes | <input type="checkbox"/> No |
| (g) Confidential handling of data | <input checked="" type="checkbox"/> Yes | <input type="checkbox"/> No |
| (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure | <input type="checkbox"/> Yes | <input checked="" type="checkbox"/> No |
5. Will Signed Consent Form be Required:
- | | | |
|--|---|--|
| (a) From subjects | <input type="checkbox"/> Yes | <input checked="" type="checkbox"/> No |
| (b) From parents or guardian (if subjects are minor) | <input checked="" type="checkbox"/> Yes | <input type="checkbox"/> 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 with 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. Example 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 Committee for review

We 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

Trainee

RESEARCH PROTOCOL

FOR OFFICE USE ONLY

Protocol No: 2000-22 Date received:
RRC Approval: Yes/ No Date: 23-08-2000
ERC Approval: Yes/No Date:

Project Title: MOLECULAR EPIDEMIOLOGY OF CRYPTOSPORIDIOSIS
Theme and key words: Persistent diarrhoea in children infected with *Cryptosporidium parvum*
Persistent diarrhoea, children, *Cryptosporidium parvum*

Principal Investigator: Wasif Ali Khan **Division:** Clinical Sciences Division **Phone:** 880-2-9886734
Address: Assistant Scientist **Email:** wakhan@icddr.org
Clinical Sciences Division, ICDDR, B
Mohakhali, Dhaka-1212

Co-Principal Investigator(s): Honorine Ward, M.D. New England Medical Center (NEMC), Boston

Co-Investigator(s): Stephen B. Calderwood, M.D & Edward T. Ryan, M.D., Mass. Gen. Hosp/Harvard Med. School, Boston; Michael Bennish, M.D. New England Medical Center (NEMC), Boston; Richard Guerrant, M.D. University of Virginia.

Student Investigator/Intern: Not Applicable

Collaborating Institute(s): New England Medical Center (NEMC), Tufts University, Boston, MA
Massachusetts General Hospital (MGH), Harvard, Boston, MA
University of Virginia, Charlottesville, VA

Population: Inclusion of special groups (Check all that apply):

- | | | |
|--------|---|--|
| Gender | <input checked="" type="checkbox"/> Male | <input type="checkbox"/> Pregnant Women |
| | <input checked="" type="checkbox"/> Females | <input type="checkbox"/> Fetuses |
| Age | <input checked="" type="checkbox"/> 0 – 5 years | <input type="checkbox"/> Prisoners |
| | <input type="checkbox"/> 5 – 9 years | <input type="checkbox"/> Destitutes |
| | <input type="checkbox"/> 10 – 19 years | <input type="checkbox"/> Service providers |
| | <input type="checkbox"/> 20 + | <input type="checkbox"/> Cognitively Impaired |
| | <input type="checkbox"/> > 65 | <input type="checkbox"/> CSW |
| | | <input type="checkbox"/> Others (specify Age) |

Project / study Site (Check all the apply):

- | | |
|--|--|
| <input checked="" type="checkbox"/> Dhaka Hospital | <input type="checkbox"/> Mirsarai |
| <input type="checkbox"/> Matlab Hospital | <input type="checkbox"/> Patyia |
| <input type="checkbox"/> Matlab DSS area | <input type="checkbox"/> Other areas in Bangladesh |
| <input type="checkbox"/> Matlab non-DSS area | <input type="checkbox"/> Outside Bangladesh |
| <input type="checkbox"/> Mirzapur | name of country: |
| <input type="checkbox"/> Dhaka Community | <input checked="" type="checkbox"/> Multi centre trial |
| <input type="checkbox"/> Chakaria | (Name other countries involved) |
| <input type="checkbox"/> Abhoynagar | USA, Brazil, RSA |

Type of Study (Check all that apply):

- | | |
|--|---|
| <input type="checkbox"/> Case Control study | <input type="checkbox"/> Cross sectional survey |
| <input type="checkbox"/> Community based trial / intervention | <input type="checkbox"/> Longitudinal Study (cohort or follow-up) |
| <input type="checkbox"/> Program Project (Umbrella) | <input type="checkbox"/> Record Review |
| <input type="checkbox"/> Secondary Data Analysis | <input type="checkbox"/> Prophylactic trial |
| <input type="checkbox"/> Clinical Trial (Hospital/Clinic) | <input type="checkbox"/> Surveillance / monitoring |
| <input type="checkbox"/> Family follow-up study | |
| <input checked="" type="checkbox"/> Others: Prospective, Observational, immune response and pathogen genotyping study. | |

Targeted Population (Check all that apply):

- | | |
|---|--------------------------------------|
| <input checked="" type="checkbox"/> No ethnic selection (Bangladeshi) | <input type="checkbox"/> Expatriates |
| <input type="checkbox"/> Bangalee | <input type="checkbox"/> Immigrants |
| <input type="checkbox"/> Tribal groups | <input type="checkbox"/> Refugee |

Consent Process (Check all that apply):

- | | |
|---|--|
| <input checked="" type="checkbox"/> Written | <input checked="" type="checkbox"/> Bengali language |
| <input type="checkbox"/> Oral | <input type="checkbox"/> English language |
| <input type="checkbox"/> None | |

Proposed Sample size: 100 (50 patients/50 controls)

Total sample size: 100

Determination of Risk: Does the Research Involve (Check all that apply):

- | | |
|---|---|
| <input type="checkbox"/> Human exposure to radioactive agents? | <input type="checkbox"/> Human exposure to infectious agents? |
| <input type="checkbox"/> Fetal tissue or abortus? | <input type="checkbox"/> Investigational new drug |
| <input type="checkbox"/> Investigational new device?
(specify _____) | <input type="checkbox"/> Existing data available via public archives/source |
| <input type="checkbox"/> Existing data available from Co-investigator | <input checked="" type="checkbox"/> Pathological or diagnostic clinical specimen only |
| | <input type="checkbox"/> Observation of public behavior |
| | <input type="checkbox"/> New treatment regime |

Yes/No

- Is the information recorded in such a manner that subjects can be identified from information provided directly or through identifiers linked to the subjects?
- Does the research deal with sensitive aspects of the subject's behavior; sexual behavior, alcohol use or illegal conduct such as drug use?
- Could the information recorded about the individual if it became known outside of the research:
- a. place the subject at risk of criminal or civil liability?
- b. damage the subject's financial standing, reputation or employability; social rejection, lead to stigma, divorce etc.

Do you consider this research (Check one):

- | | |
|--|--|
| <input type="checkbox"/> greater than minimal risk | <input type="checkbox"/> no more than minimal risk |
| <input type="checkbox"/> no risk | <input checked="" type="checkbox"/> only part of the diagnostic test |

Minimal Risk is "a risk where the probability and magnitude of harm or discomfort anticipated in the proposed research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical, psychological examinations or tests. For example, the risk of drawing a small amount of blood from a healthy individual for research purposes is no greater than the risk of doing so as a part of routine physical examination".

Yes/No

X Is the proposal funded?

If yes, sponsor Name: US NIH

Is the proposal being submitted for funding?

X If yes, name of funding agency: NIAID

Do any of the participating investigators and/or their immediate families have an equity relationship (e.g. stockholder) with the sponsor of the project or manufacturer and/or owner of the test product or device to be studied or serve as a consultant to any of the above?

IF YES, submit a written statement of disclosure to the Director.

Dates of Proposed Period of Support

(Day, Month, Year - DD/MM/YY)

Beginning date ASAP

End date 1 year

Cost Required for the Budget Period (\$)

a. Ist Year 2nd Year 3rd Year Other years

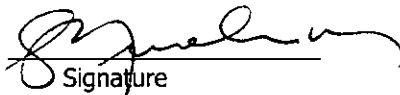
\$10,512

b. Direct Cost : 10,512 Total Cost : \$10,512

Approval of the Project by the Division Director of the Applicant

The above-mentioned project has been discussed and reviewed at the Division level as well by the external reviewers. The protocol has been revised according to the reviewer's comments and is approved.

Prof. George Fuchs
Name of the Associate Director


Signature

3/10/2000
Date of Approval

Certification by the Principal Investigator

I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

Signature of PI



Date:

03/10/2000

Name of Contact Person (if applicable)



CENTRE
FOR HEALTH AND
POPULATION RESEARCH

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH
Mail : ICDDR,B, GPO Box 128, Dhaka-1000, Bangladesh
Phone: 871751-60, Telex : 675612 ICDD BJ
Fax : 880-2-883116, 886050, 871568, 871686, Cable : Cholera Dhaka

MEMORANDUM

13 September 2000

To : Dr. Wasif Ali Khan
Clinical Sciences Division

From: Professor Mahmudur Rahman
Chairman, Ethical Review Committee (ERC)

Sub : Protocol # 2000-022

Thank you for your protocol # 2000-022 entitled "Molecular epidemiology of cryptosporidiosis" which the ERC reviewed in its meeting held on 31st August 2000. The Committee after thorough review made the following observations on your protocol:

- (a) on the face sheet item # 5B should be marked 'NO'
- (b) on the RRC face sheet Dr. Goerge Fuchs has signed in place of the PI. It is, therefore, not clear who is the PI of the protocol.

You are, therefore, advised to revise the protocol incorporating the above observations and resubmit a modified copy for consideration of the Chair.

Thank you.

cc: Associate Director
Clinical Sciences Division

ETHICAL REVIEW COMMITTEE

Minutes of the August 2000 meeting held on Thursday, 31st August 2000

The meeting started at 3:30 p.m. with Professor Mahmudur Rahman in the Chair. At the beginning of the meeting, the Chair and the members welcomed Dr. Don R. Bandaranayake, new member of the Committee in place of Dr. Lokky Wai.

Members present

Prof. Mahmudur Rahman
Dr. Don R. Bandaranayake
Prof. Hamida Akhtar Begum
Dr. AKM Iqbal Kabir
Prof. Hajera Mahtab
Mr. Mohammad Ullah

Prof. AKM Nurul Anwar
Prof. Md. Abdul Baqi
Advocate UM Habibunnesa Habiba
Prof. Jalaluddin Ashraful Haq
Dr. Nigar S. Shahid

Regret: Dr. Syeda Nahid Mukith Chowdhury, Prof. V. I. Mathan, and Prof. Barkat-e-Khuda.

Invitees : Professor David A. Sack, and Dr. W. Abdullah Brooks.

Proceedings

Agenda 1: Confirmation of the minutes of the last meeting

The minutes of the last meeting held on 26th July 2000 were confirmed with the following modification:

Agenda # 8 (protocol # 2000-015): a new paragraph (d) after the existing paragraph (c) be inserted as follows and existing paragraph # d, e, f and g be renumbered as e, f, g, and h:

" (d) A significant proportion of the vaccinated children may be at least moderately malnourished with potential impairment of their immune response and resistance to infection. The safety of this live attenuated vaccine in a malnourished population is not known. It may be potentially hazardous in such populations."

Agenda 2: Matters arising out of the minutes.

Referring to agenda # 5 and 7 (protocol # 20000-019 and 2000-017 respectively), the Chair informed that the modified copies of the protocols incorporating the observations of the Committee were approved.

Referring to Agenda # 4 (request for an addendum to protocol # 99-037), the Chair informed that the PI of the protocol had submitted a request for reconsideration of the decision of the Committee. The request would be considered today under agenda # 3.

Agenda 3: To consider the reply of Dr. M. A. Salam (for the proposal for an addendum to protocol # 99-037 entitled "Randomized, blinded trial of short-course azithromycin therapy for treatment of childhood shigellosis") in response to the decision of the Committee made in its July meeting.

The response was referred to Dr. Jalaluddin Ashraful Haq for preliminary review and comments. Dr. Haq presented his comments. After thorough review and discussion on the request, the Committee made following observations:

- (a) it is true that the omitting of 3 days course of azithromycin will reduce the cost of the study (in terms of number of patients and duration). But in case of the single dose is found ineffective, then the entire effort will go in vain. On the contrary, if the 3-day course is tested simultaneously according to the original design/protocol, and if found effective then that would add azithromycin as an alternative drug for childhood shigellosis. This is important as there are limited number of drugs available for the treatment of childhood shigellosis. This actually would reduce the cost of having two separate trials (in case of failure of the single dose regime).
- (b) though it is reported that single dose of 30 mg/kg is effective in treatment of otitis without any appreciable adverse effects in children, it may not be found to be true in infections in other anatomical sites of the body. However, the above study also recorded gastrointestinal disturbances in 17% of azithromycin treated in children. A large dose of azithromycin in a child suffering from gastroenteritis (shigellosis) may accentuate the already existing gastrointestinal disturbances.
- (c) though it is understood that it was difficult to obtain a research fund, it was believed that the cost be considered secondary to the scientific value.
- (d) while going through the protocol, it was noticed that the study would determine the pharmacokinetics of the single dose azithromycin in first 40 patients. In order to determine the serum peak and trough concentration of the drug it had been stated that 1.0 ml of blood would be collected only once at either 3, 6, 12, 24, 48 or 72 hours after the dose of the drug from each patient. How the kinetics of the drug concentration will be determined by taking single sample from separate patients? This aspect needs clarification. The timing of the collection of blood is also not correctly shown in the English version of the consent form. (p-32, 24 hours sample has not been mentioned).
- (e) no Bangla consent form was found with the protocol.

DECISION: In view of the above observations, the Committee did not find adequate justification to reconsider its earlier decision on the request for an addendum of the protocol.

Agenda 4: Protocol # 2000-022 entitled "Molecular epidemiology of cryptosporidiosis": PI: Dr. Wasif Ali Khan (CSD).

The protocol was referred to Dr. Nigar S. Shahid for her preliminary review and comments. Dr. Shahid presented her comments. After thorough review and discussion on the protocol, the Committee made the following observations:

- (a) on the face sheet item # 5B should be marked 'NO'.
- (b) on the RRC face sheet Dr. Goerge Fuchs has signed in place of the PI. It is, therefore, not clear who is the PI of the protocol.

DECISION: The Chair was authorized to approve the proposal subject to satisfactory addressing of the above observations.

Agenda 5: Proposal for an addendum to protocol # 99-033 entitled "Phase II safety and immunogenicity studies of the *enterotoxigenic Escherichia coli* (ETEC) vaccine in Bangladeshi children": PI: Dr. Firdausi Qadri (LSD).

The protocol was referred to Dr. Jalaluddin Ashraful Haq for his preliminary review and comments. Dr. Haq presented his comments in the meeting. the Committee after thorough review and discussion on the proposal made the following observations:

Considering the report of the Safety Monitoring Committee it was felt that the vaccine in stated dose was not suitable for the children of 6-17 months age. Now, to deliver the vaccine in different lower doses (i.e. lower concentration) in groups of children does not have logical reasons. The dose of vaccine to be administered should have already been optimized by the previous studies. This optimum dose of 2×10^{10} bacteria which is immunogenic must have been determined previously by studying different dose concentration. Therefore, it was necessary to know the results of those past studies. So to study different dose schedule in a Phase II study can not be recommended as such unless the results of the previous studies on different dose (con. of bacteria) are made available. Since the PI intends to see whether buffer is an offending agent or not it was also important to know how the buffer concentration was optimized previously.

DECISION: The present study involving 6-17 years aged children could, therefore, not be approved. The PI was requested to provide above information for consideration of the Committee.

Agenda 6: Protocol # 2000-023 entitled "ICDDR,B emergency Research Programme to enhance Dengue prevention and control programmes in Bangladesh": PI: Dr. Robert Breiman.

Professor David A. Sack presented before the Committee the background of the protocol and explained the compelling circumstances under which the protocol was submitted for consideration of the ERC in relaxation of the normal procedure.

The protocol was referred to Dr. AKM Iqbal Kabir who presented his comments in the meeting. Subsequently, Dr. W. Abdullah Brooks (since Dr. Breiman, the PI, was out of the country) was requested to attend the meeting for providing clarifications of the issues raised by the Committee. After thorough discussion, the Committee made the following observations on the protocol:

- (a) on the face sheet, item # 3(a) and (c) should be marked "YES".
- (b) in the consent form, it is not clearly mentioned as to how many times, the blood samples would be collected.
- (c) there should be standardized treatment protocol for the management of patients with dengue fever identified/diagnosed either in the hospital or in the community.
- (d) there is no indication that there exists any understanding of co-operation between Dhaka Medical College Hospital, Shishu Hospital, IEDCR of Directorate General of health Services. Unless suitable personnel from these hospitals, and IEDCR are included as co-investigators, it may be difficult to carry out the work and attain the objectives.
- (e) a consent form for hospitalized patients is needed since blood samples will be collected from the hospitalised patients.
- (f) for clinical management studies involving evaluation of treatment strategies, full pledged research protocol detailing all the activities will be required to enable the ERC to consider ethical issues involved.
- (g) for Community based activities surveillance for epidemiological studies, selection of Kamlapur Urban Slum raised several queries:
 - why an urban slum at the first place, why not a more affluent post area? If its trend is that mosquitoes may move from affluent areas to slum at some stage (as PI tried to explain), why not include both urban posh area and a slum.
 - what happens, if sufficient sample to draw valid conclusion are not obtained in Kamlapur?
 - active involvement IEDCR (for DGHS) for Community based Surveillance may be necessary for them to carry on the surveillance later on (page # 4 of the protocol is referred).

- standard treatment protocol for community based patients will also be needed.
- (h) budget allocation for hospital based surveillance seems to be disproportionate since hospital records will be utilized and there already exists a surveillance system and regular reporting to DGHS from these hospitals. Given what already exists in the hospital system for surveillance, the budget of US\$171,823 seems high but until a detailed breakdown is available it is not possible to be certain. The budget of US\$197,379 for the community-based surveillance component similarly needs clarification.
 - (i) budget re-allocation may be considered to extend the 'Community based active surveillance' sites.
 - (j) the researchers propose to "*implement a multifaceted, integrated program to provide solid scientific bases for designing and evolving public health strategies for prevention and control dengue in Bangladesh*".(p2) This is a noteworthy aim which unfortunately is not fully supported by the proposed research program, although the results may contribute to such a outcome.
 - (k) the hospital-based surveillance component will only focus on three selected hospitals in Dhaka that currently deal with most of the cases of dengue in the capital. Bearing in mind that there is already a hospital case recording and reporting system in place, the additional demographic, clinical, and laboratory data that the research effort will gather (at considerable cost) will not appreciably enhance the capacity of the MOHFW to mount a "rapid response" to the dengue outbreak. The identification of "case-associated locations" and using established methods for vector elimination is already possible.
 - (l) the establishment of a (hospital) *sentinel surveillance system* will as stated require enrolment of hospitals from all parts of the country at some future date. It is not clear whether the research program will conduct this expansion during the inter-epidemic period, and if so whether that will incur a further cost. What may be feasible is the setting up of a pilot surveillance system in Dhaka (again according to the budget at considerable cost). What is required urgently is a rapid response system from at least the major hospitals in the whole country to be established and some hands-on training of personnel for this purpose.
 - (m) the epidemiology study will unfortunately not give any information about risk factors including risk behaviours from the non-slum areas where many cases have occurred. Furthermore the selection of controls for the nested case-control study from the same slum area as the cases may not elicit useful information about environmental conditions.

- (n) the mosquito vector surveillance component may contribute to what is documented and utilized already for outbreak prediction, as will the viral detection component. The potential public health benefit of the latter component is obscure given what is known already.
- (o) the serological assays (Component V) forms a critical part of the research program that has great potential. The study population for this component needs elucidation. There seems to be some overlap here with the clinical management component in terms of blood sampling and antibody detection.
- (p) the immunologic response and host characteristics component of the program (Component VI) also needs patient numbers and selection criteria. This component too will have some overlap with the clinical management component as far as blood sampling from hospital patients is concerned.
- (q) the baseline questionnaire should include questions about the type of dwelling ie materials used for construction as well as the availability of lighting (electricity, paraffin etc). It should also inquire about general mosquito control measures already taken eg City Council action, unblocking of stagnant drains etc.
- (r) there is a major ethical issue regarding the "opportunistic" interventions of authorities in any so-called emergency situation. Most countries will have legislation that control such actions with powers vested on the "proper authorities". Even under these urgent circumstances, the use of invasive procedures e.g., blood sampling does require clearance from ethics committees unless such procedures are routinely done under normal (non-emergency) situations. Such clearance may be given.

DECISION: The PI was advised to address the above issues and resubmit a modified copy of the protocol for consideration of the Committee.

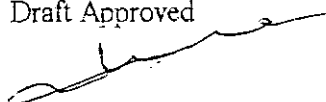
Agenda 7: Any other business

It was resolved that the Committee will meet again late this week or early next week as soon as the response of the PI of the protocol # 2000-023 is received.

The meeting ended at 5:45 p.m. with a vote of thanks to the Chair.

3 September 2000

Draft Approved


Professor Mahmudur Rahman
Chairman
Ethical Review Committee

Confirmed

Chairperson, ERC

(FACE SHEET)

ETHICAL REVIEW COMMITTEE, ICDDR,B.

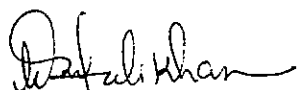
Principal Investigator: Wasif Ali Khan
 Application No. 2000-22
 Title of Study: Molecular Epidemiology of
 Cryptosporidiosis

Trainee Investigator (if any): _____
 Supporting Agency (if Non-ICDDR,B) _____
 Project Status: _____
 New Study
 Continuation with change
 No change (do not fill out rest of the form)

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

1. Source of Population:
- | | | |
|---|---|--|
| (a) Ill subjects | <input checked="" type="checkbox"/> Yes | <input type="checkbox"/> No |
| (b) Non-ill subjects | <input type="checkbox"/> Yes | <input checked="" type="checkbox"/> No |
| (c) Minor or persons under guardianship | <input checked="" type="checkbox"/> Yes | <input type="checkbox"/> No |
2. Does the Study Involve:
- | | | |
|---|---|--|
| (a) Physical risk to the subjects | <input type="checkbox"/> Yes | <input checked="" type="checkbox"/> No |
| (b) Social risk | <input type="checkbox"/> Yes | <input checked="" type="checkbox"/> No |
| (c) Psychological risks to subjects | <input type="checkbox"/> Yes | <input checked="" type="checkbox"/> No |
| (d) Discomfort to subjects | <input checked="" type="checkbox"/> Yes | <input type="checkbox"/> No |
| (e) Invasion of privacy | <input type="checkbox"/> Yes | <input checked="" type="checkbox"/> No |
| (f) Disclosure of information damaging to subject or others | <input type="checkbox"/> Yes | <input checked="" type="checkbox"/> No |
3. Does the Study Involve:
- | | | |
|--|------------------------------|--|
| (a) Use of records (hospital, medical, death or other) | <input type="checkbox"/> Yes | <input checked="" type="checkbox"/> No |
| (b) Use of fetal tissue or abortus | <input type="checkbox"/> Yes | <input checked="" type="checkbox"/> No |
| (c) Use of organs or body fluids | <input type="checkbox"/> Yes | <input checked="" type="checkbox"/> No |
4. Are Subjects Clearly Informed About:
- | | | |
|--|---|--|
| (a) Nature and purposes of the study | <input checked="" type="checkbox"/> Yes | <input type="checkbox"/> No |
| (b) Procedures to be followed including alternatives used | <input checked="" type="checkbox"/> Yes | <input type="checkbox"/> No |
| (c) Physical risk | <input checked="" type="checkbox"/> Yes | <input type="checkbox"/> No |
| (d) Sensitive questions | <input type="checkbox"/> Yes | <input checked="" type="checkbox"/> No |
| (e) Benefits to be derived | <input checked="" type="checkbox"/> Yes | <input type="checkbox"/> No |
| (f) Right to refuse to participate or to withdraw from study | <input checked="" type="checkbox"/> Yes | <input type="checkbox"/> No |
| (g) Confidential handling of data | <input checked="" type="checkbox"/> Yes | <input type="checkbox"/> No |
| (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure | <input type="checkbox"/> Yes | <input checked="" type="checkbox"/> No |
5. Will Signed Consent Form be Required:
- | | | |
|--|---|-----------------------------|
| (a) From subjects | <input checked="" type="checkbox"/> Yes | <input type="checkbox"/> No |
| (b) From parents or guardian (if subjects are minor) | <input checked="" type="checkbox"/> Yes | <input type="checkbox"/> No |
6. Will precautions be taken to protect anonymity of subjects
- | | |
|---|-----------------------------|
| <input checked="" type="checkbox"/> Yes | <input type="checkbox"/> No |
|---|-----------------------------|
7. Check documents being submitted herewith to Committee:
- Umbrella proposal - Initially submit an with 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. Example 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 Committee for review

We 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

 Trainee

Principal Investigator: Last, first, middle: Khan, Wasif, Ali

ICDDR, Centre for Health & Population Research RRC APPLICATION FORM

RESEARCH PROTOCOL

FOR OFFICE USE ONLY

Protocol No: 2000-22 Date received:
RRC Approval: Yes/ No Date:
ERC Approval: Yes/No Date:

Project Title: MOLECULAR EPIDEMIOLOGY OF CRYPTOSPORIDIOSIS
Theme and key words: Persistent diarrhoea in children infected with *Cryptosporidium parvum*
Persistent diarrhoea, children, *Cryptosporidium parvum*

Principal Investigator: Wasif Ali Khan **Division:** Clinical Sciences **Division Phone:** 880-2-9886734
Address: Assistant Scientist **Email:** wakhan@icddr.org
Clinical Sciences Division, ICDDR, B
Mohakhali, Dhaka-1212

Co-Principal Investigator(s): Honorine Ward, M.D. New England Medical Center (NEMC), Boston

Co-Investigator(s): Stephen B. Calderwood, M.D & Edward T. Ryan, M.D., Mass. Gen. Hosp/Harvard Med. School, Boston; Michael Bennis, M.D. New England Medical Center (NEMC), Boston; Richard Guerrant, M.D. University of Virginia.

Student Investigator/Intern: Not Applicable

Collaborating Institute(s): New England Medical Center (NEMC), Tufts University, Boston, MA
Massachusetts General Hospital (MGH), Harvard, Boston, MA
University of Virginia, Charlottesville, VA

Population: Inclusion of special groups (Check all that apply):

- | | |
|--|--|
| Gender | <input type="checkbox"/> Pregnant Women |
| X Male | <input type="checkbox"/> Fetuses |
| X Females | <input type="checkbox"/> Prisoners |
| Age | <input type="checkbox"/> Destitutes |
| X 0 - 5 years | <input type="checkbox"/> Service providers |
| <input type="checkbox"/> 5 - 9 years | <input type="checkbox"/> Cognitively Impaired |
| <input type="checkbox"/> 10 - 19 years | <input type="checkbox"/> CSW |
| <input type="checkbox"/> 20 + | <input type="checkbox"/> Others (specify Age) |
| <input type="checkbox"/> > 65 | |

Project / study Site (Check all the apply):

- | | |
|--|--|
| X Dhaka Hospital | <input type="checkbox"/> Mirsarai |
| <input type="checkbox"/> Matlab Hospital | <input type="checkbox"/> Patyia |
| <input type="checkbox"/> Matlab DSS area | <input type="checkbox"/> Other areas in Bangladesh |
| <input type="checkbox"/> Matlab non-DSS area | <input type="checkbox"/> Outside Bangladesh |
| <input type="checkbox"/> Mirzapur | name of country: |
| <input type="checkbox"/> Dhaka Community | X Multi centre trial |
| <input type="checkbox"/> Chakaria | (Name other countries involved) |
| <input type="checkbox"/> Abhoynagar | USA, Brazil, RSA |

Type of Study (Check all that apply):

- | | |
|--|---|
| <input type="checkbox"/> Case Control study | <input type="checkbox"/> Cross sectional survey |
| <input type="checkbox"/> Community based trial / intervention | <input type="checkbox"/> Longitudinal Study (cohort or follow-up) |
| <input type="checkbox"/> Program Project (Umbrella) | <input type="checkbox"/> Record Review |
| <input type="checkbox"/> Secondary Data Analysis | <input type="checkbox"/> Prophylactic trial |
| <input type="checkbox"/> Clinical Trial (Hospital/Clinic) | <input type="checkbox"/> Surveillance / monitoring |
| <input type="checkbox"/> Family follow-up study | |
| X Others: Prospective, Observational, immune response and pathogen genotyping study. | |

Targeted Population (Check all that apply):

- | | |
|---|--------------------------------------|
| <input checked="" type="checkbox"/> No ethnic selection (Bangladeshi) | <input type="checkbox"/> Expatriates |
| <input type="checkbox"/> Bangalee | <input type="checkbox"/> Immigrants |
| <input type="checkbox"/> Tribal groups | <input type="checkbox"/> Refugee |

Consent Process (Check all that apply):

- | | |
|---|--|
| <input checked="" type="checkbox"/> Written | <input checked="" type="checkbox"/> Bengali language |
| <input type="checkbox"/> Oral | <input type="checkbox"/> English language |
| <input type="checkbox"/> None | |

Proposed Sample size: 100 (50 patients/50 controls)

Total sample size: 100

Determination of Risk: Does the Research Involve (Check all that apply):

- | | |
|---|---|
| <input type="checkbox"/> Human exposure to radioactive agents? | <input type="checkbox"/> Human exposure to infectious agents? |
| <input type="checkbox"/> Fetal tissue or abortus? | <input type="checkbox"/> Investigational new drug |
| <input type="checkbox"/> Investigational new device?
(specify _____) | <input type="checkbox"/> Existing data available via public archives/source |
| <input type="checkbox"/> Existing data available from Co-investigator | <input checked="" type="checkbox"/> Pathological or diagnostic clinical specimen only |
| | <input type="checkbox"/> Observation of public behavior |
| | <input type="checkbox"/> New treatment regime |

Yes/No

- Is the information recorded in such a manner that subjects can be identified from information provided directly or through identifiers linked to the subjects?
- Does the research deal with sensitive aspects of the subject's behavior; sexual behavior, alcohol use or illegal conduct such as drug use?

Could the information recorded about the individual if it became known outside of the research:

- a. place the subject at risk of criminal or civil liability?
- b. damage the subject's financial standing, reputation or employability; social rejection, lead to stigma, divorce etc.

Do you consider this research (Check one):

- | | |
|--|--|
| <input type="checkbox"/> greater than minimal risk | <input type="checkbox"/> no more than minimal risk |
| <input type="checkbox"/> no risk | <input checked="" type="checkbox"/> only part of the diagnostic test |

Minimal Risk is "a risk where the probability and magnitude of harm or discomfort anticipated in the proposed research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical, psychological examinations or tests. For example, the risk of drawing a small amount of blood from a healthy individual for research purposes is no greater than the risk of doing so as a part of routine physical examination".

Table of Contents

	Page Numbers
Face Page	1
Project Summary	5
Description of the Research Project	
Hypothesis to be tested	6
Specific Aims	6
Background of the Project Including Preliminary Observations	7
Research Design and Methods	11
Facilities Available	14
Data Analysis	15
Use of Animals	16
Literature Cited	17
Dissemination and Use of Findings	23
Collaborative Arrangements	24
Biography of the Investigators	25
Detailed Budget	32
Budget Justifications	33
Other Support	34
Ethical Assurance: Protection of Human Rights	36
Appendix	
Consent Forms in English	38
Consent Forms in Bangla	40
Abstract summary for the Ethical Review Committee	42

Check here if appendix is included

Principal Investigator: Last, first, middle: Khan, Wasif, Ali

PROJECT SUMMARY: Describe in concise terms, the hypothesis, objectives, and the relevant background of the project. Describe concisely the experimental design and research methods for achieving the objectives. This description will serve as a succinct and precise and accurate description of the proposed research is required. This summary must be understandable and interpretable when removed from the main application. (TYPE TEXT WITHIN THE SPACE PROVIDED).

Principal Investigator
Wasif Ali Khan

Project Name
MOLECULAR EPIDEMIOLOGY AND HUMORAL IMMUNE RESPONSES TO *CRYPTOSPORIDIUM PARVUM*

Total Budget: US\$10,512 Beginning Date: Ending Date: One Year from study Initiation

Cryptosporidial infection is mostly asymptomatic and almost always self-limiting in immunocompetent hosts, but may be severe and life threatening in immunocompromised patients such as malnourished children and those with acquired immunodeficiency syndrome (AIDS). Cryptosporidial infection in early childhood may result in subsequent impairment in growth, physical fitness and cognitive function.

The Diarrhoea Treatment Centre of the ICDDR, B in Dhaka, Bangladesh annually cares for more than 100,000 persons with diarrhea, 80% of whom are children ≤ 5 years of age. Previous studies have found that 2% of these children have *C. parvum* present in the stool. The objective of this study is to perform genotyping, serum and fecal antibody analysis on *C. parvum*-infected and uninfected children and correlate with clinical findings.

We will identify *C. parvum*-infected children by screening stools by microscopy using a modified acid fast stain of all eligible children who come to the ICDDR, B. Those infected with *C. parvum* will be asked to participate in the study. Control children without *C. parvum* infection will be identified as the next age appropriate child who comes to the Treatment Centre.

A questionnaire will be prepared to note (CRF - case report form) the routine history and physical findings, parents of the children will be asked about drinking water source, contact with livestock, pets and other animals. Stool samples (10-50 ml) will be collected and transported as soon as possible in portable cool boxes to the laboratory. *C. parvum* oocysts in stool samples will be identified using a modified acid fast stain. Stools from study subjects will be divided into two aliquots. One will be stored in 2.5% potassium dichromate at 4°C. The other half will be stored frozen at -80°C. Approximately (1ml of blood) will be obtained from *C. parvum* infected children and control patients at the time of presentation. Serum will be stored at -80°C.

All samples will be shipped to NEMC, Tufts at the end of patient recruitment. Stool samples positive for *C. parvum* by microscopy will be analyzed for isolate genotype at NEMC. To analyze genotypic diversity, restriction fragment length polymorphisms (RFLP) analysis of PCR-derived DNA fragments (PCR-RFLP) will be used. We will also evaluate serum IgG, IgM and IgA and fecal sIgA responses by ELISA and immunoblot analysis to *C. parvum* antigens in children with *C. parvum* infection, as well as in age and sex-matched controls.

We will identify 50 children ≤ 5 years of age infected with *C. parvum* infection. An equal number of children as control with diarrhea but without *C. parvum* infection will also be enrolled. These children will be age-matched in the following categories - < 12 months; 13-24 months; 25-60 months. The inclusion criteria are: age 15d to 60 months, either sex, duration of illness ≥ 14 days, and written informed consent obtained from the guardians of the study patient. Patients with chronic renal, cardiac, or hepatic problems will be excluded from enrolment into the study.

Principal Investigator: Last, first, middle: Khan, Wasif, Ali

KEY PERSONNEL (List names of all investigators including PI and their respective specialties)

Name	Professional Discipline/ Specialty	Role in the Project
1. Wasif Ali Khan	Internal Medicine	Principal Investigator
2. Honorine Ward	Internal Medicine	Co-PI
3. Stephen B. Calderwood	Infectious Disease	Investigator
4. Edward T. Ryan	Infectious Disease	Investigator
5. Michael Bennish	Infectious Disease	Investigator
6. Richard Guerrant	Infectious Disease	Investigator

DESCRIPTION OF THE RESEARCH PROJECT

Hypothesis to be tested:

Concisely list in order, in the space provided, the hypothesis to be tested and the Specific Aims of the proposed study. Provide the scientific basis of the hypothesis, critically examining the observations leading to the formulation of the hypothesis.

Specific Aims:

Describe the specific aims of the proposed study. State the specific parameters, biological functions/ rates/ processes that will be assessed by specific methods (**TYPE WITHIN LIMITS**).

Please note: This study is funded by the Opportunity Pool of Funds of the International Collaborations in Infectious Disease Research (ICIDR) program of the U.S. National Institute of Allergy and Infectious Diseases (NIAID). This pool of funding is available to Pathogen Specific Groups (PSG) of the ICIDRs. Three ICIDRs comprise the Enteric Pathogen Specific Group (Harvard-Massachusetts General Hosp./ICDDR B Dhaka, Bangladesh; Tufts-NEMC/Republic of South Africa; University of Virginia/Brazil). This award is separate from the ICIDR projects.

The overall goal of this pilot study is to investigate the molecular epidemiology of and host antibody response to *Cryptosporidium parvum* in three areas of the developing world where this parasite is a major cause of persistent diarrhea in children with and without HIV infection. Cryptosporidial infection in early childhood may result in subsequent impairment in growth, physical fitness and cognitive function. In children with AIDS, this parasite may cause severe and often fatal diarrheal disease for which there is no specific therapy. Very little is known about the molecular epidemiology of *C. parvum* or about host antibody responses to this parasite, particularly in the setting of the developing world. The interaction between ICIDR investigators in this pathogen specific group presents a unique opportunity to study these aspects of *C. parvum* infection in Bangladesh, Brazil and rural S. Africa as representatives of developing countries in three continents and brings together the interests and expertise of each of the groups. For a number of years the UVA/Brazil ICIDR has studied the association of cryptosporidiosis with persistent diarrhea and the long-term effects of *C. parvum* infection on physical fitness and cognitive function in children. A major goal of the Tufts-NEMC/S. Africa ICIDR is to study the molecular epidemiology of *Cryptosporidium parvum* infection in HIV-infected and uninfected children. The focus of the MGH-Harvard/Bangladesh ICIDR is to study mucosal immune responses, in particular to *Vibrio cholerae*. This pilot study will provide important information about *C. parvum* genotypes and antibody responses to *C. parvum* and their clinical and epidemiological correlates in each of the three countries. This will enable us to determine whether these parameters can be used as tools to study the epidemiology of cryptosporidiosis in developing countries in future studies. The long term goal is to develop interventional strategies to minimize or prevent transmission of this disease.

The specific aims are:

1. Molecular epidemiology of *C. parvum* infection:

Principal Investigator: Last, first, middle: Khan, Wasif, Ali

- a. To determine the genotypes of isolates from children with *C. parvum* infection in Brazil and Bangladesh and compare genotype information with that obtained from S. Africa (already being performed as part of Tufts-NEMC/S. Africa ICIDR)
- b. To correlate *C. parvum* genotype information with geographic location, age, clinical status (duration and severity of illness), drinking water source and contact with animals.

2. Seroepidemiology and systemic and mucosal antibody responses to *C. parvum* antigens

- a. To determine the presence and titer of serum IgG, IgM and IgA and fecal secretory (s) IgA anti-*C. parvum* antibodies (in particular antibodies to the 15/17 kDa immunodominant antigen) by ELISA and immunoblot in children infected with *C. parvum* and in controls in Brazil, Bangladesh and S. Africa.
- b. To correlate serum and fecal antibody status with age and clinical status (duration and severity of illness) in children in the three countries.

Background of the Project including Preliminary Observations

Describe the relevant background of the proposed study. Discuss the previous related works on the subject by citing specific references. Describe logically how the present hypothesis is supported by the relevant background observations including any preliminary results that may be available. Critically analyze available knowledge in the field of the proposed study and discuss the questions and gaps in the knowledge that need to be fulfilled to achieve the proposed goals. Provide scientific validity of the hypothesis on the basis of background information. If there is no sufficient information on the subject, indicate the need to develop new knowledge. Also include the **significance and rationale** of the proposed work by specifically discussing how these accomplishments will bring benefit to human health in relation to biomedical, social, and environmental perspectives. (DO NOT EXCEED 5 PAGES, USE CONTINUATION SHEETS).

The intestinal protozoan parasite *Cryptosporidium parvum*, is a significant cause of diarrheal disease worldwide (26, 29). Cryptosporidial infection is often asymptomatic and almost always self-limiting in immunocompetent hosts, but may be severe and life threatening in immunocompromised patients such as those with acquired immunodeficiency syndrome (AIDS). In developing countries *C. parvum* is a major cause of persistent or chronic diarrhea in children with and without HIV infection (12, 36, 37, 42, 47, 65). In countries such as Brazil, Peru and Guinea-Bissau, West Africa, cryptosporidial infection in childhood has been reported to be associated with subsequent impairment in growth, physical fitness and cognitive function (1, 16, 28, 41). *Cryptosporidium* is highly infectious with an ID50 as low as 132 oocysts (19). The infection may be transmitted by direct person to person spread or by ingestion of contaminated food or water. *C. parvum* has been identified as the causative agent of numerous water borne outbreaks of diarrheal disease (20). To date there is no specific therapy approved for treatment of cryptosporidiosis.

C. parvum is a major pathogen in children with acute as well as persistent diarrhea in the three countries represented by the ICIDRs in this pathogen specific group. Investigators in the UVA/Brazil ICIDR have studied cryptosporidiosis in children with persistent diarrhea in Brazil for a numbers of years. A 4-year cohort study in an urban slum in northeastern Brazil, conducted by these investigators, detected *C. parvum* infection in 7.4% of all stools tested from children with diarrhea (48). Of these, *C. parvum* was identified in 16.5% of stools from children with persistent diarrhea, compared to 8.4% from children with acute diarrhea and 4.0 % of children without diarrhea. A recent report by this group of a 45 month prospective cohort study of persistent diarrhea in children in a shantytown in northeast Brazil confirmed that *Cryptosporidium* was a major pathogen and was significantly associated with 11.4% of episodes of acute diarrheal illness and 23.9% of persistent diarrhea episodes (37). Studies from this group have also shown that cryptosporidial infection in early childhood may result in subsequent impairment in growth, physical fitness and cognitive function (1, 28). Most of the children in these cohort studies were not HIV-infected (Guerrant, R. L., unpublished). However, a recent study of hospitalized HIV-infected patients with diarrhea showed that *Cryptosporidium* was a significant pathogen (Guerrant, R. L., unpublished). Cryptosporidiosis is also an important cause of diarrhea in children in Bangladesh (86). A study from the ICDDR, B (the MGH-Harvard/Bangladesh ICIDR site) reported the presence of *C. parvum* in the stools of 3.5 % of 1949 children with acute diarrhea (5). Another recent case-control study from the same site confirmed that *C. parvum* is significantly associated with diarrheal illness in children in Dhaka (2). Most of the children in the ICDDR, B studies are also not HIV-infected (Khan W. A., unpublished). In S. Africa, *Cryptosporidium* is a major pathogen associated with persistent diarrhea in children with AIDS. A recent study by investigators from the Tufts-NEMC/S. Africa ICIDR

Principal Investigator: Last, first, middle: Khan, Wasif, Ali

showed that *Cryptosporidium* was the major pathogen identified in HIV-infected children with persistent diarrhea and was present in stool samples from 24.8% of 101 of these children (64).

Molecular epidemiology of cryptosporidiosis

Very little is known about the epidemiology of cryptosporidiosis, particularly in developing countries. Molecular analyses of *C. parvum* isolates have identified genetic differences among these isolates leading to a molecular approach to study the epidemiology of this infection (6, 39, 43, 44, 51, 52, 60, 63, 66, 72, 81, 83). Human isolates of *C. parvum* have been classified into two genetically distinct genotypes, a human-type (genotype 1), present mainly in human infections, and a bovine or calf type (genotype 2), present in both animal and human hosts (51, 54, 66, 81). Genotypic variation has been demonstrated at various genetic loci including the ITS1 region of rDNA (10, 44), the SSU rRNA gene (84), polythreonine ORF (11), DHFR-TS gene (80), COWP gene (52, 68), EF-2 gene, TRAP C1 gene (67, 69), TRAP C2 gene (54) elongation factor-2-gene (31) and beta-tubulin gene (7, 63, 71). These studies did not identify heterogeneity within the two genotypes. However, recent studies have shown that significant intragenotypic variation can be identified at certain genetic loci. A recent analysis of microsatellite repeats reported that the human genotype can be further differentiated into 2 sub-genotypes and the calf genotype into 4 sub-genotypes (6). Some sub-genotypes showed a wide geographic distribution, whereas others were restricted to specific geographic locations. Investigators from the Tufts-NEMC/S. Africa ICIDR and others recently cloned and sequenced a *C. parvum* gene encoding surface glycoproteins gp40/46 and gp15/17 (13, 57, 70). Sequence analysis of calf and human genotype isolates at this locus revealed that this gene is highly polymorphic (49, 70) and that single nucleotide and single amino acid polymorphisms in these sequences defined at least 5 distinct allelic groupings, one of which is a calf or genotype 2 allele and the others human or genotype 1 alleles (70). In future studies, analysis of the single nucleotide polymorphisms at this locus may be useful for fingerprinting *C. parvum* isolates for detailed molecular epidemiological studies.

At the present time however, the significance of the genetic heterogeneity in *C. parvum* isolates is not clear and it remains to be determined whether there is a correlation between genotype and clinical or epidemiological correlates such as age, HIV status, duration and severity of clinical illness, geographic location, drinking water source and contact with animals. Some studies have suggested a correlation of parasite genotype with infectivity in mice, clinical illness in animal models as well as clinical features in AIDS patients. For instance, the majority of AIDS patients enrolled in ACTG trial 336 were found to be infected with *C. parvum* isolates of the human genotype (83). Isolates of this genotype were not infectious in neonatal mice. Another study of 140 AIDS patients with cryptosporidiosis reported the presence of both human and bovine as well as mixed genotype infections (17). In several patients in this study, isolates of the human genotype were associated with less severe clinical disease. A recent study reported that a human genotype isolate propagated in piglets displayed phenotypic differences from calf genotype isolates (82). This isolate was not infective for interferon knockout mice, had a shorter oocyst survival time and caused milder clinical illness in piglets than did calf genotype isolates. In a recent survey of published data on 215 individuals with cryptosporidiosis and known HIV status, 75% of *C. parvum* isolates were of the human genotype (74). There was, however, no significant difference in genotype pattern between 29 HIV-infected (90% human genotype) persons, and 187 HIV-uninfected persons (84% human genotype). The majority (96% of 95) of isolates from two waterborne outbreaks of cryptosporidiosis in the UK were reported to be of the human genotype (53). However, a study of persons with sporadic diarrhea associated with cryptosporidiosis in the UK found that 62% of 194 isolates were of the calf genotype (39). In this study there was no significant difference in distribution of genotype by sex or age. There was, however, a significant difference in genotype by place of residence within the UK, and by history of recent foreign travel

The majority of genotyping studies performed thus far have been on *C. parvum* isolates from developed countries. Very little information is available on genotypes of isolates from developing countries. Our preliminary data (see Preliminary studies below) on genotyping of *C. parvum* isolates from HIV-infected children with persistent diarrhea in South Africa indicate that the human isolate is predominant although both genotypes are present. Similarly our preliminary data on genotyping of *C. parvum* isolates from Brazil indicate that the human genotype is more prevalent.

Seroepidemiology and antibody responses to *C. parvum* antigens

The host immune response to *C. parvum* and mechanisms of protective immunity are not well understood. Both cell-mediated and humoral responses are believed to be important in protective immunity (62, 73). A number of studies have reported the presence of *C. parvum*-specific serum IgG, IgM and IgA and fecal IgA antibodies by ELISA, IFA or immunoblot analysis in humans following cryptosporidial infection (8, 9, 18, 21-25, 27, 30, 33, 34, 38,

Principal Investigator: Last, first, middle: Khan, Wasif, Ali

40, 45, 46, 50, 56, 58, 61, 62, 75-79, 85). The role of antibodies in resistance to infection and protective immunity is not clear. However, in many of these studies the presence of *C. parvum*-specific antibodies has been used as an indicator of past or present cryptosporidial infection. Seroprevalence studies have reported a wide range of seropositivity depending on age, geographic location, living and environmental conditions. Investigators from the UVA/Brazil ICIDR found that by 2 years of age almost all children in a semiurban impoverished community in Fortaleza acquired serum antibody, compared to ~50% among children in Anhui, a rural area in China and 16.9% of children in Virginia, USA (85). There is no information on antibody responses to *C. parvum* in children from Bangladesh or S. Africa.

In order to develop more specific and sensitive assays for the presence of serum and mucosal antibodies recent studies have focused on specific *C. parvum* antigens. Immunoblot analyses have identified the presence of antibodies to a wide range of antigens ranging from <14 to >200 kDa in M_r (62). Of these, antigens 15/17 kDa in M_r have been consistently identified (9, 21, 22, 24, 30, 38, 45, 46, 61). The majority of these studies have been performed in developed countries. Very little information is known about antibody responses to the immunodominant 15/17 kDa antigen in developing countries. In a preliminary study conducted by investigators from the Tufts-NEMC/S. Africa ICIDR of a cohort of Bedouin children in southern Israel with and without cryptosporidial infection, sera from infected children were also found to consistently display antibodies to a 15 kDa protein (32). Till recently, crude oocyst antigen preparations were used for these analyses. A recent study measured serum IgG responses to a partially purified native preparation of the 15/17 kDa protein by ELISA (58). This ELISA was more specific and sensitive than ELISAs currently in use, which employ crude oocyst antigen. Investigators in the Tufts-NEMC/S. Africa ICIDR and others have recently cloned the gene encoding the 15/17 kDa immunodominant antigen, also named gp15 or Cp17 (13, 57, 70). Sera from *C. parvum*-infected individuals which reacted with the native protein also reacted with recombinant Cp17 (57). Investigators in the Tufts-NEMC/S. Africa ICIDR have also expressed the recombinant 15kDa protein as a thioredoxin fusion protein in *E. coli* (13) and purified the fusion protein by nickel affinity chromatography. Preliminary studies indicate that sera from *C. parvum*-infected individuals display reactivity to the recombinant protein by immunoblotting (Jaison, S. and Ward, H. D., unpublished). In collaboration with Cynthia Chappell and Sara Dann, University of Texas School of Public Health, Houston, TX, ELISAs to measure serum and fecal antibodies to gp15 using this recombinant fusion protein as antigen have been standardized. Studies are currently underway to evaluate serum and fecal antibody responses to gp15 (using these ELISAs) in ongoing studies of *C. parvum*-infected volunteers (14, 15, 19, 50).

PRELIMINARY STUDIES

A. Genotypic analysis of *C. parvum* isolates from HIV-infected children with persistent diarrhea in Durban, S. Africa.

As part of an ongoing study to determine the prevalence of the enteric pathogens associated with persistent diarrhea in HIV-infected children in South Africa, we undertook a genotypic analysis of isolates from *C. parvum*-infected children (35). Blood and stool samples were collected from HIV-infected children 6 to 36 m presenting with diarrhea to King Edward VIII Hospital, Durban, S. Africa (64). Children with diarrhea lasting more than 7 days (before or during the hospital stay) were enrolled in the study. Stool samples were processed for detection of enteric bacterial, viral and protozoal pathogens.

Results:

Clinical:

The mean age of 101 patients enrolled to date was 14.9m. Study children were a mean 64% of median weight-for-age. Median HIV RNA was 1.197×10^6 copies/ml (range: 502-21.9 $\times 10^6$ copies/ml) and CD4 count was 726×10^3 /ml (range 12-2,880). *C. parvum* was the major pathogen identified and was present in 25 (24.8%) children. *C. parvum* infected children had significantly lower CD4 counts (g.mean: 299 vs. 648×10^3 /ml, $p=0.002$) and CD4:CD8 ratio (g.mean: 0.195 vs. 0.366, $p=0.004$) than other children. *C. parvum*-infected children did not differ from other children in age, weight-for-age or plasma HIV RNA. Significantly more *C. parvum*-infected children died within 8 weeks of admission than children who were not infected (6/16 vs. 7/68, $p = 0.007$).

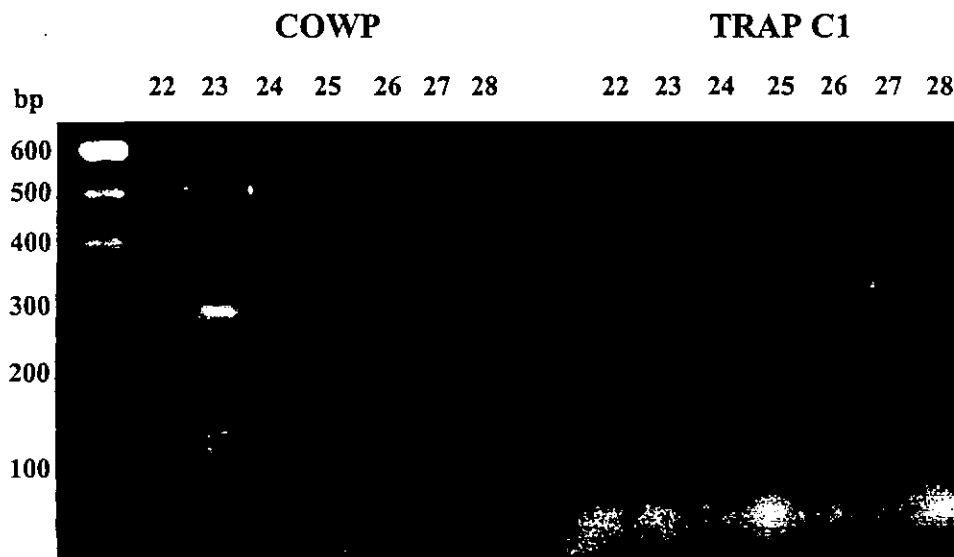
Figure 1: Genotypic Analysis:

Fig 1: PCR RFLP analysis of *C. parvum* isolates at the COWP and TRAP C1 loci. Isolate 25 is of the calf genotype (genotype 2) and the others are of the human genotype (genotype 1).

Stool specimens from the *C. parvum*-infected children were stored at -20°C and shipped to New England Medical Center, Boston on dry ice for genotyping.

Table 1: Genotypic analysis of *C. parvum* isolates from S. Africa

Study No	Age (mths)	Sex	Diarrhea (days) ¹	CD4 count (x 10 ³ /mL)	HIV RNA copies/mL	Clinical outcome ²	<i>C. parvum</i> in stool	PCR TRAP C1 COWP	RFLP TRAP C1 COWP
14	15	M	12	1904	630374	ALIVE	+	+	C
15	22	M	8	298	1955587	NK	+	+	H
16	26	F	14	464	1357759	ALIVE	+	+	C
17	8	F	7	855	11623833	DEAD	+	+	H
18	9	M	8	146	1597611	DEAD	+	-	-
19	13	F	7	424	22309	DEAD	+	+	H
20	6	M	7	172	2284562	ALIVE	+	+	H
21	8	F	3	1240	1675577	ALIVE	+	+	C
22	12	F	14	418	1678333	ALIVE	+	+	H
23	6	M	60	24	882880	DEAD	+	+	H
24	10	M	30	1134	1922891	ALIVE	+	+	H
25	21	M	7	344	5796283	ALIVE	+	+	C
26	14	M	14	410	1927915	ALIVE	+	+	H
27	12	M	7	628	1224586	ALIVE	+	+	H
28	7	F	7	1932	186235	ALIVE	+	+	H
29	25	F	5	352	617534	ALIVE	+	+	H
30	12	F	8	848	1004918	ALIVE	+	+	H
31	34	M	21	60	1575402	DEAD	+	+	C
32	6	F	29	598	1550000	NK	+	+	H
33	17	M	14	138	2413618	ALIVE	+	-	-
34	7	M	30	1776	1028245	ALIVE	+	-	-
35	8	M	7	39	2377872	DEAD	+	+	H
36	19	F	14	1070	1878336	ALIVE	+	+	H
37	11	F	15	ND	1077402	DEAD	+	+	H

- ¹Duration of diarrhea in days prior to admission.
- ²Clinical outcome = alive or dead by 8 weeks post admission/recruitment ,
- NK= Not Known, as defaulted follow up, ND = Not done, as child died before enrollment.
- H= human or genotype 1, C= calf or genotype 2

Principal Investigator: Last, first, middle: Khan, Wasif, Ali

DNA was extracted from these samples by slight modification of a previously described method (39). This DNA was used to amplify fragments of the *Cryptosporidium* oocyst wall protein (COWP) and the thrombospondin-related adhesive protein of *Cryptosporidium* (TRAP C1) genes by PCR using previously published primers (67, 68). DNA fragments could be amplified in 21 of 24 samples. Restriction length polymorphism with the restriction enzyme *Rsa*I was then used to determine the genotype of *C. parvum* isolates (Fig 1). The results are shown in Table 1. 16 of 21(76%) isolates were determined to be of the human genotype at both loci. Association between genotype and various clinical parameters was determined using Mann Whitney and Fishers exact tests. Analysis of the results showed that there was no significant association between genotype and duration of diarrhea, CD4 count, HIV RNA, sex or clinical outcome. Presence of the calf genotype was associated with older age (mean, 20.8 vs. 11.9 months, $p=0.05$). This is the first report of genotyping of *C. parvum* isolates from Africa. In this population of HIV infected children with diarrhea in S. Africa, the human genotype of *C. parvum* was more prevalent than the calf genotype. In this study the numbers of isolates (particularly the calf genotype) studied was small. Analysis of larger numbers of isolates may reveal significant associations of genotype with various clinical and epidemiological (not analyzed in this study) parameters.

Research Design and Methods

Describe in detail the methods and procedures that will be used to accomplish the objectives and specific aims of the project. Discuss the alternative methods that are available and justify the use of the method proposed in the study. Justify the scientific validity of the methodological approach (biomedical, social, or environmental) as an investigation tool to achieve the specific aims. Discuss the limitations and difficulties of the proposed procedures and sufficiently justify the use of them. Discuss the ethical issues related to biomedical and social research for employing special procedures, such as invasive procedures in sick children, use of isotopes or any other hazardous materials, or social questionnaires relating to individual privacy. Point out safety procedures to be observed for protection of individuals during any situations or materials that may be injurious to human health. The methodology section should be sufficiently descriptive to allow the reviewers to make valid and unambiguous assessment of the project. **(DO NOT EXCEED TEN PAGES, USE CONTINUATION SHEETS).**

1. Study Design

Exploratory study

2. Selection of Patients for the study

A total of 50 children ≤ 5 years of age infected with *C. parvum* (where pediatric HIV-infections are uncommon) will be enrolled for the study. An equal number ($N = 50$) of children with diarrhea but not infected with *C. parvum* will be identified and taken as control patients. These children will be age-matched in the following categories - < 12 months; 13-24 months; 25-60 months.

The sample size of 50 per cell gives us the ability to detect differences between groups of half a standard deviation for continuous variables and 25% differences for categorical variables, as well as insuring that we can detect correlations as small as 0.4, all at the 0.05 level of significance with 80% power. It should be noted that this is an exploratory study, in part directed to estimating the variability within these groups so that we can be sure that future studies are powered adequately.

Inclusion Criteria

Age	15d to 60 months
Gender	Either sex
Duration of illness	≥ 14 days

Written informed consent for participation in the study from their guardian.

Exclusion Criteria

Patients with chronic renal, cardiac, or hepatic problems.

Note: Patients will be enrolled at any time of the day, and on any day of the year, subject to fulfillment of enrollment criteria.

Principal Investigator: Last, first, middle: Khan, Wasif, Ali

Bangladesh: The Diarrhoea Treatment Centre of the ICDDR,B in Dhaka, Bangladesh annually cares for more than 100,000 persons with diarrhea, 80% of whom are children \leq 5 years of age. Previous studies have found that 1.4-3.5 % of these children have *C. parvum* present in the stool(2, 5). Incidence of *C. parvum* among persistent diarrhoea children was 8.8% (5). Out of average 200 children a day those coming to the Treatment Centre, ICDDR,B 5% are persistent diarrhoea children. Thus we expect to get 1-2 patients with persistent diarrhoea that will be infected with *C. parvum* per week. We will identify *C. parvum*-infected children by screening stools of all eligible children who come to the outpatient clinic by microscopy using a modified acid fast stain. Those who are found to be infected with *C. parvum* will be asked to participate in the study. Parents will be asked for informed consent. Control children without *C. parvum* infection will be identified as the next age appropriate child who comes to the Treatment Centre who does not have *C. parvum* identified in their stool. Should parents decline to have their child participate in the study, the next age-matched child will be identified and asked to participate. Since pediatric HIV-infections in this population are extremely uncommon, HIV testing will not be performed. All study participants will have signed informed consent as approved by the Bangladesh as well as MGH-Harvard Human Investigation Committees and by NIH.

Brazil: Stools and serum will be obtained from our "banked" specimens, as well as from children in our prospective studies in Goncalves Dias that are getting underway (HIV infection in these children is not an issue as we know and have followed closely all the mothers over many years). In addition, we shall obtain stools and serum from patients with AIDS in the Statewide Infectious Diseases hospital in Fortaleza. This will permit an assessment of the predominant strains of *Cryptosporidium* seen in our past studies as well as in our present studies in the community as well as in hospitalized patients with diarrhea and AIDS. In addition, we will obtain age-matched neighborhood controls from the same population for fecal and serum antibody studies. All study participants will have signed informed consent as approved by the Brazilian as well as University of Virginia Human Investigation Committees and by NIH.

S. Africa: Children for study will be identified at two sites. One is the King Edward VIII Hospital in Durban, which is the largest acute care hospital in KwaZulu/Natal province, and cares for ~1000 children with diarrhea per year. The other is children enrolled in a prospective study of enteric infections in children with and without HIV-infection in rural KwaZulu/Natal province – a cohort that will come from the core of the parent ICIDR study. As in Bangladesh, control children at King Edward VIII Hospital will be selected by identifying the next age-matched child coming to the outpatient department who is not HIV-infected. In the field study in rural KwaZulu/Natal control patients will be selected by identifying the child with the next sequential study number enrolled in the study who is not *C. parvum* infected, and who fits into the age matching. All study participants will have signed informed consent as approved by the S. Africa as well as Tufts-NEMC Human Investigation Committees and by NIH.

Sample collection:

STOOL

Stool samples (10-50 ml) will be collected and transported as soon as possible in portable cool boxes to the laboratory. *C. parvum* oocysts in stool samples will be identified using a modified acid fast stain. Stools from study subjects will be divided into two aliquots. One will be stored in 2.5% potassium dichromate at 4°C. The other half will be stored frozen at -80°C.

BLOOD

1ml of blood will be obtained from *C. parvum* infected children and control patients at the time of presentation. Serum will be stored at -80°C.

At the end of all patient recruitment samples will be shipped to Boston for genotyping and antibody studies at NEMC.

Genotyping:

Stool samples positive for *C. parvum* by microscopy will be analyzed for isolate genotype. We will analyze genotypic diversity using restriction fragment length polymorphisms (RFLP) analysis of PCR-derived DNA fragments (PCR-RFLP). For these analyses, four previously identified polymorphic loci will first be amplified using PCR followed by restriction enzyme digests to reveal polymorphisms in these genes. Oocysts will be purified from fecal samples stored in 2.5% potassium dichromate at 4°C by salt flotation and Percoll density gradient centrifugation (3) or by immunomagnetic separation (55) and DNA will be extracted as previously described (83). Alternatively, DNA will be directly extracted from "banked" frozen stool samples as described in Preliminary studies using a modification of a

Principal Investigator: Last, first, middle: Khan, Wasif, Ali

previously described method (39). Genotyping will be performed as described previously by PCR-RFLP of the TRAP C1 gene (67), COWP gene (68), polythreonine locus (11), and gp40/15 gene (70). The genotype of isolates from *C. parvum*-infected HIV-positive and negative children will be compared and correlated with epidemiological and clinical data. Comparison between isolates from HIV-positive and HIV-negative children will be confined to S. Africa and hospitalized children in Brazil, since pediatric HIV infections are very uncommon in Bangladesh and in the children from the cohort studies in Goncalves Dias, Brazil.

Serum and fecal antibody response:

We will evaluate serum IgG, IgM and IgA and fecal sIgA responses to *C. parvum* antigens in children with *C. parvum* infection as well as in age and sex-matched controls by ELISA and immunoblot analysis. For both analyses we will use a crude oocyst preparation as well as the recombinant 15 kDa protein as antigen.

Serum ELISA: Isotype-specific, anti-*C. parvum* serum antibodies will be quantitated by ELISA as previously described (4). Briefly, 96-well plates will be coated with a freeze-thaw lysate of *C. parvum* oocysts or recombinant 15 kDa protein. Non-specific binding will be blocked with 1% bovine serum albumin (BSA) in PBS for 1 hour at 37°C. Wells will be incubated with serum diluted in 1% BSA in PBS for 1 hour at 37°C. After washing three times with 0.05% Tween-20 in PBS (PBS-T), wells will be incubated with alkaline phosphatase-conjugated goat-anti-human heavy chain-specific IgG, IgA or IgM diluted in 1% BSA/PBS for 1 hour at 37°C. After washing three times with PBS-T, wells will be incubated with substrate and absorbance read.

Fecal ELISA: To quantitate anti-*C. parvum* fecal sIgA the aliquot of stool frozen at -80°C will be thawed and processed as described previously (59). Briefly, 4G of stool will be added to 16 ml of PBS-T containing protease inhibitors. The suspension will be filtered through cheesecloth and centrifuged at 20,000 x g for 30 min. The supernatant will be frozen in aliquots at -80°C after addition of 0.1% BSA and 0.02% sodium azide. Total fecal sIgA will be quantitated by ELISA and stool supernatants adjusted to the same concentration of total sIgA, before quantitating specific sIgA. Briefly, 96 well plates will be coated with goat anti-human IgA in carbonate buffer pH 9.6. Non-specific binding will be blocked with 5% fetal bovine serum (FBS) in PBS-T for 5 hour at RT. Wells will then be incubated with serial dilutions of fecal supernatants in 5% FBS in PBS-T overnight at 4°C. After washing three times with PBS-T, wells will be incubated with a mouse monoclonal antibody to human sIgA diluted in 5% FBS in PBS-T for 1 hour at 37°C, washed three times and incubated with goat anti-mouse IgG-biotin conjugate in 5% FBS in PBS-T for 1 hr at 37°C. After three washes, wells will then be incubated with Streptavidin-horse radish peroxidase (HRP) in 5% FBS in PBS-T for 1 hr at 37°C, washed three times, incubated with substrate and absorbance read. The amount of total sIgA will be quantitated by comparison to known amounts of purified human sIgA. *C. parvum*-specific sIgA in fecal supernatants containing a fixed amount of total sIgA will be quantitated by ELISA as described above except that crude oocyst preparation or recombinant 15kDa protein will be used as antigens to coat the plates.

Immunoblot analysis: The presence of antibodies to specific *C. parvum* antigens will be determined by immunoblot analysis as described previously. Briefly, a freeze-thaw lysate of *C. parvum* oocysts or recombinant 15 kDa protein will be separated by 5-15% gradient SDS PAGE and transferred to nitrocellulose. Non-specific binding will be blocked with 10% NGS in 10 mM Tris-HCl, 150 mM sodium chloride, pH 8.0 (TBS) for 1 h before incubation with serum or fecal supernatant (containing a fixed amount of total IgA) diluted in 5% NGS in 0.1% Tween 20 in TBS (0.1% T-TBS) for 90 min at RT. After washing three times with 0.1% T-TBS, strips will be incubated with horseradish peroxidase conjugated goat anti-human IgG, IgA or IgM (sIgA for fecal supernatant) antibody diluted in 5% NGS-0.1% T-TBS for 1 h at RT. Strips will be washed, incubated in a chemiluminescence substrate, exposed to film and developed.

The presence of serum and fecal antibodies to *C. parvum* antigens, including the immunodominant 15/17 kDa antigen in *C. parvum*-infected children from each of the three countries will be compared and correlated with genotype information, as well as epidemiological and clinical data. Again, comparison of antibody status in HIV-positive and HIV-negative children will be confined to S. Africa and hospitalized patients in Fortaleza, Brazil, since pediatric HIV infections are very uncommon in Bangladesh and in the children from the cohort studies in Goncalves Dias, Brazil.

Facilities Available

Describe the availability of physical facilities at the place where the study will be carried out. For clinical and laboratory-based studies, indicate the provision of hospital and other types of patient's care facilities and adequate laboratory support. Point out the laboratory facilities and major equipments that will be required for the study. For field studies, describe the field area including its size, population, and means of communications. (TYPE WITHIN THE PROVIDED SPACE).

Site: Clinical Research and service centre, Dhaka hospital, ICDDR, B.

Study population: The Diarrhoea Treatment Centre of the ICDDR,B in Dhaka, Bangladesh annually cares for more than 100,000 persons with diarrhea, 80% of whom are children ≤ 5 years of age. Previous studies have found that 1.4-3.5 % of these children have *C. parvum* present in the stool(2, 5). Thus on average 200 children a day come to the Treatment Centre, 2-7 of whom can be expected to be infected with *C. parvum*. We will identify *C. parvum*-infected children by screening stools of all eligible children who come to the outpatient clinic by microscopy using a modified acid fast stain. Those who are found to be infected with *C. parvum* will be asked to participate in the study. Control children will be identified as the next age appropriate child who comes to the Treatment Centre who does not have *C. parvum* identified in their stool.

Laboratory facilities: The clinical laboratory service of the Laboratory Sciences Division of ICDDR, B will be used for routine testing.

Data Analysis

Describe plans for data analysis. Indicate whether data will be analyzed by the investigators themselves or by other professionals. Specify what statistical softwares packages will be used and if the study is blinded, when the code will be opened. For clinical trials, indicate if interim data analysis will be required to monitor further progress of the study. (TYPE WITHIN THE PROVIDED SPACE).

Statistical methods

Data will be entered onto computer using SPSS for Windows (Version 8.0). Range checks and a double-entry system will be used to insure the validity of data entry. The significance of differences in continuous variables (like age, duration of illness, serum antibody titer etc.) will be assessed by Student's t test for data that are normally distributed, or by Mann-Whitney U test for data, which are not normally distributed. Significance of differences in the proportions (severity of illness, source of drinking water, animal contact etc.) will be assessed by the chi-square test, and Fisher's exact test will be employed when the expected number in any cell in the comparison is less than 5.

Use of Animals

Describe in the space provided the type and species of animal that will be used in the study. Justify with reasons the use of particular animal species in the experiment and the compliance of the animal ethical guidelines for conducting the proposed procedures.

Animal studies will not be undertaken as part of this protocol.

Literature Cited

Identify all cited references to published literature in the text by number in parentheses. List all cited references sequentially as they appear in the text. For unpublished references, provide complete information in the text and do not include them in the list of Literature Cited. There is no page limit for this section, however exercise judgment in assessing the "standard" length.

1. **Agnew, D. G., A. A. Lima, R. D. Newman, T. Wuhib, R. D. Moore, R. L. Guerrant, and C. L. Sears.** 1998. Cryptosporidiosis in northeastern Brazilian children: association with increased diarrhea morbidity. *J Infect Dis.* **177**(3):754-60.
2. **Albert, M. J., A. S. Faruque, S. M. Faruque, R. B. Sack, and D. Mahalanabis.** 1999. Case-control study of enteropathogens associated with childhood diarrhea in Dhaka, Bangladesh. *J Clin Microbiol.* **37**(11):3458-64.
3. **Arrowood, M. J., and C. R. Sterling.** 1987. Isolation of *Cryptosporidium* oocysts and sporozoites using discontinuous sucrose and isopycnic Percoll gradients. *J Parasitol.* **73**(2):314-9.
4. **Baecher-Allan, C. M., K. Santora, S. Sarantopoulos, W. Den, S. R. Sompuram, J. Sharon, A. M. Cevallos, N. Bhat, and H. Ward.** 1999. Generation of a polyclonal fab phage display library to the protozoan parasite *cryptosporidium parvum* [In Process Citation]. *Comb Chem High Throughput Screen.* **2**(6):319-25.
5. **Bhattacharya, M. K., T. Teka, A. S. Faruque, and G. J. Fuchs.** 1997. Cryptosporidium infection in children in urban Bangladesh. *J Trop Pediatr.* **43**(5):282-6.
6. **Caccio, S., W. Homan, R. Camilli, G. Traldi, T. Kortbeek, and E. Pozio.** 2000. A microsatellite marker reveals population heterogeneity within human and animal genotypes of *cryptosporidium parvum*. *Parasitology.* **120**(Pt 3):237-44.
7. **Caccio, S., W. Homan, K. van Dijk, and E. Pozio.** 1999. Genetic polymorphism at the beta-tubulin locus among human and animal isolates of *Cryptosporidium parvum* [In Process Citation]. *FEMS Microbiol Lett.* **170**(1):173-9.
8. **Campbell, P. N., and W. L. Current.** 1983. Demonstration of serum antibodies to *Cryptosporidium* sp. in normal and immunodeficient humans with confirmed infections. *J Clin Microbiol.* **18**(1):165-9.
9. **Caputo, C., A. Forbes, F. Frost, M. I. Sinclair, T. R. Kunde, J. F. Hoy, and C. K. Fairley.** 1999. Determinants of antibodies to *Cryptosporidium* infection among gay and bisexual men with HIV infection. *Epidemiol Infect.* **122**(2):291-7.
10. **Carraway, M., S. Tzipori, and G. Widmer.** 1996. Identification of genetic heterogeneity in the *Cryptosporidium parvum* ribosomal repeat. *Appl Environ Microbiol.* **62**(2):712-6.
11. **Carraway, M., S. Tzipori, and G. Widmer.** 1997. A new restriction fragment length polymorphism from *Cryptosporidium parvum* identifies genetically heterogeneous parasite populations and genotypic changes following transmission from bovine to human hosts. *Infect Immun.* **65**(9):3958-60.
12. **Cegielski, J. P., Y. R. Ortega, S. McKee, J. F. Madden, L. Gaido, D. A. Schwartz, K. Manji, A. F. Jorgensen, S. E. Miller, U. P. Pulipaka, A. E. Msengi, D. H. Mwakyusa, C. R. Sterling, and L. B. Reller.** 1999. *Cryptosporidium*, enterocytozoon, and cyclospora infections in pediatric and adult patients with diarrhea in Tanzania. *Clin Infect Dis.* **28**(2):314-21.
13. **Cevallos, A. M., X. Zhang, M. Waldor, S. Jaison, S. Tzipori, X. Zhou, M. Neutra, and H. D. Ward.** 2000. Molecular cloning and expression of a gene encoding *Cryptosporidium parvum* glycoproteins gp40 and gp15. *Infect. Immun.* **in press.**

Principal Investigator: Last, first, middle: Khan, Wasif, Ali

14. **Chappell, C. L., P. C. Okhuysen, C. R. Sterling, and H. L. DuPont.** 1996. *Cryptosporidium parvum*: intensity of infection and oocyst excretion patterns in healthy volunteers. *J Infect Dis.* **173**(1):232-6.
15. **Chappell, C. L., P. C. Okhuysen, C. R. Sterling, C. Wang, W. Jakubowski, and H. L. Dupont.** 1999. Infectivity of *Cryptosporidium parvum* in healthy adults with pre-existing anti-*C. parvum* serum immunoglobulin G. *Am J Trop Med Hyg.* **60**(1):157-64.
16. **Checkley, W., L. D. Epstein, R. H. Gilman, R. E. Black, L. Cabrera, and C. R. Sterling.** 1998. Effects of *Cryptosporidium parvum* infection in Peruvian children: growth faltering and subsequent catch-up growth. *Am J Epidemiol.* **148**(5):497-506.
17. **Clark, D., M. Lane, and M. Molecular Parasitology Meeting. Woodshole.** 1998. Genetic diversity and virulence of *Cryptosporidium* isolates. Molecular Parasitology Meeting. Woodshole, MA,.
18. **Cozon, G., F. Biron, M. Jeannin, D. Cannella, and J. P. Revillard.** 1994. Secretory IgA antibodies to *Cryptosporidium parvum* in AIDS patients with chronic cryptosporidiosis. *J Infect Dis.* **169**(3):696-9.
19. **DuPont, H. L., C. L. Chappell, C. R. Sterling, P. C. Okhuysen, J. B. Rose, and W. Jakubowski.** 1995. The infectivity of *Cryptosporidium parvum* in healthy volunteers. *N Engl J Med.* **332**(13):855-9.
20. **Fricker, C. R., and J. H. Crabb.** 1998. Water-borne cryptosporidiosis: detection methods and treatment options. *Adv Parasitol.* **40**:241-78.
21. **Frost, F. J., R. L. Calderon, T. B. Muller, M. Curry, J. S. Rodman, D. M. Moss, and A. A. de la Cruz.** 1998. A two-year follow-up survey of antibody to *Cryptosporidium* in Jackson County, Oregon following an outbreak of waterborne disease. *Epidemiol Infect.* **121**(1):213-7.
22. **Frost, F. J., and G. F. Caun.** 1998. Serologic response to human *Cryptosporidium* infections [letter]. *Infect Immun.* **66**(8):4008-9.
23. **Frost, F. J., A. A. de la Cruz, D. M. Moss, M. Curry, and R. L. Calderon.** 1998. Comparisons of ELISA and Western blot assays for detection of *Cryptosporidium* antibody. *Epidemiol Infect.* **121**(1):205-11.
24. **Frost, F. J., T. Muller, G. F. Craun, D. Fraser, D. Thompson, R. Notenboom, and R. L. Calderon.** 2000. Serological analysis of a cryptosporidiosis epidemic [In Process Citation]. *Int J Epidemiol.* **29**(2):376-9.
25. **Gomez Morales, M. A., E. Pozio, and G. P. Croppo.** 1991. Detection of *Cryptosporidium* circulating antigens in human and calf sera. *J Protozool.* **38**(6):182S-183S.
26. **Griffiths, J. K.** 1998. Human cryptosporidiosis: epidemiology, transmission, clinical disease, treatment, and diagnosis. *Adv Parasitol.* **40**:37-85.
27. **Groves, V. J., D. Lehmann, and G. L. Gilbert.** 1994. Seroepidemiology of cryptosporidiosis in children in Papua New Guinea and Australia. *Epidemiol Infect.* **113**(3):491-9.
28. **Guerrant, D. I., S. R. Moore, A. A. Lima, P. D. Patrick, J. B. Schorling, and R. L. Guerrant.** 1999. Association of early childhood diarrhea and cryptosporidiosis with impaired physical fitness and cognitive function four-seven years later in a poor urban community in northeast Brazil. *Am J Trop Med Hyg.* **61**(5):707-13.
29. **Guerrant, R. L.** 1997. Cryptosporidiosis: an emerging, highly infectious threat. *Emerg Infect Dis.* **3**(1):51-7.
30. **Isaac-Renton, J., J. Blatherwick, W. R. Bowie, M. Fyfe, M. Khan, A. Li, A. King, M. McLean, L. Medd, W. Moorehead, C. S. Ong, and W. Robertson.** 1999. Epidemic and endemic seroprevalence of antibodies to *Cryptosporidium* and *Giardia* in residents of three communities with different drinking water supplies. *Am J Trop Med Hyg.* **60**(4):578-83.

Principal Investigator: Last, first, middle: Khan, Wasif, Ali

31. **Jones, D. E., T. D. Tu, S. Mathur, R. W. Sweeney, and D. P. Clark.** 1995. Molecular cloning and characterization of a *Cryptosporidium parvum* elongation factor-2 gene. *Mol Biochem Parasitol.* **71**(1):143-7.
32. **Kelley M, Hamer DH, Keusch GT, Dagan R, Fraser D, and W. HD.** Manuscript in preparation. Humoral immune response to *Cryptosporidium parvum* in a cohort of Bedouin children followed from birth to two years of age. .
33. **Kuhls, T. L., D. A. Mosier, D. L. Crawford, and J. Griffis.** 1994. Seroprevalence of cryptosporidial antibodies during infancy, childhood, and adolescence. *Clin Infect Dis.* **18**(5):731-5.
34. **Laxer, M. A., A. K. Alcantara, M. Javato-Laxer, D. M. Menorca, M. T. Fernando, and C. P. Ranoa.** 1990. Immune response to cryptosporidiosis in Philippine children. *Am J Trop Med Hyg.* **42**(2):131-9.
35. **Leav, B., N. Rollins, M. Pent, G. Kindra, T. Kasambira, W. Sturm, D. Steele, P. Skolnik, M. Bennish, and H. Ward.** 2000. Genotypic analysis of *Cryptosporidium parvum* isolates from HIV-infected children in South Africa. abstract 1682 submitted to the 49th Annual Meeting of the American Society of Tropical Medicine and Hygiene, Houston, TX.
36. **Lima, A. A., G. Fang, J. B. Schorling, L. de Albuquerque, J. F. McAuliffe, S. Mota, R. Leite, and R. L. Guerrant.** 1992. Persistent diarrhea in northeast Brazil: etiologies and interactions with malnutrition. *Acta Paediatr Suppl.* **381**:39-44.
37. **Lima, A. A., S. R. Moore, M. S. Barboza Jr, A. M. Soares, M. A. Schlepner, R. D. Newman, C. L. Sears, J. P. Nataro, D. P. Fedorko, T. Wuhib, J. B. Schorling, and R. L. Guerrant.** 2000. Persistent Diarrhea Signals a Critical Period of Increased Diarrhea Burdens and Nutritional Shortfalls: A Prospective Cohort Study among Children in Northeastern Brazil. *J Infect Dis.* **181**(5):1643-1651.
38. **McLauchlin, J., D. P. Casemore, S. Moran, and S. Patel.** 1998. The epidemiology of cryptosporidiosis: application of experimental sub- typing and antibody detection systems to the investigation of water- borne outbreaks. *Folia Parasitol.* **45**(2):83-92.
39. **McLauchlin, J., S. Pedraza-Diaz, C. Amar-Hoetzeneder, and G. L. Nichols.** 1999. Genetic characterization of *Cryptosporidium* strains from 218 patients with diarrhea diagnosed as having sporadic cryptosporidiosis. *J Clin Microbiol.* **37**(10):3153-8.
40. **Mead, J. R., M. J. Arrowood, and C. R. Sterling.** 1988. Antigens of *Cryptosporidium* sporozoites recognized by immune sera of infected animals and humans. *J Parasitol.* **74**(1):135-43.
41. **Molbak, K., M. Andersen, P. Aaby, N. Hojlyng, M. Jakobsen, M. Sodemann, and A. P. da Silva.** 1997. *Cryptosporidium* infection in infancy as a cause of malnutrition: a community study from Guinea-Bissau, west Africa. *Am J Clin Nutr.* **65**(1):149-52.
42. **Molbak, K., N. Hojlyng, A. Gottschau, J. C. Sa, L. Ingholt, A. P. da Silva, and P. Aaby.** 1993. Cryptosporidiosis in infancy and childhood mortality in Guinea Bissau, west Africa. *Bmj.* **307**(6901):417-20.
43. **Morgan, U., R. Weber, L. Xiao, I. Sulaiman, R. C. Thompson, W. Ndiritu, A. Lal, A. Moore, and P. Deplazes.** 2000. Molecular characterization of *Cryptosporidium* isolates obtained from human immunodeficiency virus-infected individuals living in Switzerland, Kenya, and the United States. *J Clin Microbiol.* **38**(3):1180-3.
44. **Morgan, U. M., P. Deplazes, D. A. Forbes, F. Spano, H. Hertzberg, K. D. Sargent, A. Elliot, and R. C. Thompson.** 1999. Sequence and PCR-RFLP analysis of the internal transcribed spacers of the rDNA repeat unit in isolates of *Cryptosporidium* from different hosts. *Parasitology.* **118**(Pt 1):49-58.
45. **Moss, D. M., S. N. Bennett, M. J. Arrowood, S. P. Wahlquist, and P. J. Lammie.** 1998. Enzyme-linked immunoelectrotransfer blot analysis of a cryptosporidiosis outbreak on a United States Coast Guard cutter. *Am J Trop Med Hyg.* **58**(1):110-8.

Principal Investigator: Last, first, middle: Khan, Wasif, Ali

46. **Moss, D. M., C. L. Chappell, P. C. Okhuysen, H. L. DuPont, M. J. Arrowood, A. W. Hightower, and P. J. Lammie.** 1998. The antibody response to 27-, 17-, and 15-kDa *Cryptosporidium* antigens following experimental infection in humans. *J Infect Dis.* **178(3):**827-33.
47. **Mwachari, C., B. I. Batchelor, J. Paul, P. G. Waiyaki, and C. F. Gilks.** 1998. Chronic diarrhoea among HIV-infected adult patients in Nairobi, Kenya. *J Infect.* **37(1):**48-53.
48. **Newman, R. D., C. L. Sears, S. R. Moore, J. P. Nataro, T. Wuhib, D. A. Agnew, R. L. Guerrant, and A. A. Lima.** 1999. Longitudinal study of *Cryptosporidium* infection in children in northeastern Brazil. *J Infect Dis.* **180(1):**167-75.
49. **O'Connor, R. M., A. M. Cevallos, S. Tzipori, and H. D. Ward.** 2000. Expression of *Cryptosporidium parvum* glycoproteins gp40 and gp15. abstract submitted to Molecular Parasitology Meeting XI, Woodshole, MA, Spetemebr 2000.
50. **Okhuysen, P. C., C. L. Chappell, C. R. Sterling, W. Jakubowski, and H. L. DuPont.** 1998. Susceptibility and serologic response of healthy adults to reinfection with *Cryptosporidium parvum*. *Infect Immun.* **66(2):**441-3.
51. **Ong, C. S., D. L. Eisler, S. H. Goh, J. Tomblin, F. M. Awad-El-Kariem, C. B. Beard, L. Xiao, I. Sulaiman, A. Lal, M. Fyfe, A. King, W. R. Bowie, and J. L. Isaac-Renton.** 1999. Molecular epidemiology of cryptosporidiosis outbreaks and transmission in British Columbia, Canada. *Am J Trop Med Hyg.* **61(1):**63-9.
52. **Patel, S., S. Pedraza-Diaz, and J. McLauchlin.** 1999. The identification of *Cryptosporidium* species and *Cryptosporidium parvum* directly from whole faeces by analysis of a multiplex PCR of the 18S rRNA gene and by PCR/RFLP of the *Cryptosporidium* outer wall protein (COWP) gene. *Int J Parasitol.* **29(8):**1241-7.
53. **Patel, S., S. Pedraza-Diaz, J. McLauchlin, and D. P. Casemore.** 1998. Molecular characterisation of *Cryptosporidium parvum* from two large suspected waterborne outbreaks. Outbreak Control Team South and West Devon 1995, Incident Management Team and Further Epidemiological and Microbiological Studies Subgroup North Thames 1997. *Commun Dis Public Health.* **1(4):**231-3.
54. **Peng, M. M., L. Xiao, A. R. Freeman, M. J. Arrowood, A. A. Escalante, A. C. Weltman, C. S. Ong, W. R. Mac Kenzie, A. A. Lal, and C. B. Beard.** 1997. Genetic polymorphism among *Cryptosporidium parvum* isolates: evidence of two distinct human transmission cycles. *Emerg Infect Dis.* **3(4):**567-73.
55. **Pereira, M. D., E. R. Atwill, and T. Jones.** 1999. Comparison of sensitivity of immunofluorescent microscopy to that of a combination of immunofluorescent microscopy and immunomagnetic separation for detection of *Cryptosporidium parvum* oocysts in adult bovine feces. *Appl Environ Microbiol.* **65(7):**3236-9.
56. **Petry, F.** 1998. Epidemiological study of *Cryptosporidium parvum* antibodies [corrected] in sera of persons from Germany [published erratum appears in *Infection* 1998 Mar-Apr;26(2):115]. *Infection.* **26(1):**7-10.
57. **Priest, J. W., J. P. Kwon, M. J. Arrowood, and P. J. Lammie.** 2000. Cloning of the immunodominant 17-kDa antigen from *Cryptosporidium parvum*. *Mol Biochem Parasitol.* **106(2):**261-71.
58. **Priest, J. W., J. P. Kwon, D. M. Moss, J. M. Roberts, M. J. Arrowood, M. S. Dworkin, D. D. Juranek, and P. J. Lammie.** 1999. Detection by enzyme immunoassay of serum immunoglobulin G antibodies that recognize specific *Cryptosporidium parvum* antigens. *J Clin Microbiol.* **37(5):**1385-92.
59. **Qadri, F., G. Jonson, Y. A. Begum, C. Wenneras, M. J. Albert, M. A. Salam, and A. M. Svennerholm.** 1997. Immune response to the mannose-sensitive hemagglutinin in patients with cholera due to *Vibrio cholerae* O1 and O0139. *Clin Diagn Lab Immunol.* **4(4):**429-34.

Principal Investigator: Last, first, middle: Khan, Wasif, Ali

60. **Quiroz, E. S., C. Bern, J. R. MacArthur, L. Xiao, M. Fletcher, M. J. Arrowood, D. K. Shay, M. E. Levy, R. I. Glass, and A. Lal.** 2000. An outbreak of cryptosporidiosis linked to a foodhandler. *J Infect Dis.* **181**(2):695-700.
61. **Reperant, J. M., M. Naciri, S. Iochmann, M. Tilley, and D. T. Bout.** 1994. Major antigens of *Cryptosporidium parvum* recognised by serum antibodies from different infected animal species and man. *Vet Parasitol.* **55**(1-2):1-13.
62. **Riggs, M. W.** 1997. Immunology: Host Response and Development of Passive Immunotherapy and Vaccines. *In R. Fayer (ed.), Cryptosporidium and Cryptosporidiosis.* CRC Press.
63. **Rochelle, P. A., E. M. Jutras, E. R. Atwill, R. De Leon, and M. H. Stewart.** 1999. Polymorphisms in the beta-tubulin gene of *Cryptosporidium parvum* differentiate between isolates based on animal host but not geographic origin. *J Parasitol.* **85**(5):986-9.
64. **Rollins, N. C., M. Pent, G. Kindra, T. Kasambira, W. Sturm, D. Steele, P. R. Skolnik, and M. Bennish.** 2000. CD4 count, HIV viral load, and enteric pathogens in HIV-infected children with persistent diarrhea in Durban, South Africa. abstract, accepted for presentation, Pediatric Academic Societies Annual Meeting, May 2000.
65. **Sodemann, M., M. S. Jakobsen, K. Molbak, C. Martins, and P. Aaby.** 1999. Episode-specific risk factors for progression of acute diarrhoea to persistent diarrhoea in west African children. *Trans R Soc Trop Med Hyg.* **93**(1):65-8.
66. **Spano, F., L. Putignani, A. Crisanti, P. Sallicandro, U. M. Morgan, S. M. Le Blancq, L. Tchack, S. Tzipori, and G. Widmer.** 1998. Multilocus genotypic analysis of *cryptosporidium parvum* isolates from different hosts and geographical origins [In Process Citation]. *J Clin Microbiol.* **36**(11):3255-9.
67. **Spano, F., L. Putignani, S. Guida, and A. Crisanti.** 1998. *Cryptosporidium parvum*: PCR-RFLP analysis of the TRAP-C1 (thrombospondin-related adhesive protein of *Cryptosporidium-1*) gene discriminates between two alleles differentially associated with parasite isolates of animal and human origin. *Exp Parasitol.* **90**(2):195-8.
68. **Spano, F., L. Putignani, J. McLauchlin, D. P. Casemore, and A. Crisanti.** 1997. PCR-RFLP analysis of the *Cryptosporidium* oocyst wall protein (COWP) gene discriminates between *C. wrairi* and *C. parvum*, and between *C. parvum* isolates of human and animal origin. *FEMS Microbiol Lett.* **150**(2):209-17.
69. **Spano, F., L. Putignani, S. Naitza, C. Puri, S. Wright, and A. Crisanti.** 1998. Molecular cloning and expression analysis of a *Cryptosporidium parvum* gene encoding a new member of the thrombospondin family. *Mol Biochem Parasitol.* **92**(1):147-62.
70. **Strong, W. B., J. Gut, and R. G. Nelson.** 2000. Cloning, characterization and sequence polymorphism analysis of a *Cryptosporidium parvum* gene encoding a highly variable 60 kDa glycoprotein and its 15 kDa and 45 kDa zoite surface antigen products. *Infect. Immun.* **in press.**
71. **Sulaiman, I. M., A. A. Lal, M. J. Arrowood, and L. Xiao.** 1999. Biallelic polymorphism in the intron region of beta-tubulin gene of *Cryptosporidium* parasites. *J Parasitol.* **85**(1):154-7.
72. **Sulaiman, I. M., L. Xiao, C. Yang, L. Escalante, A. Moore, C. B. Beard, M. J. Arrowood, and A. A. Lal.** 1998. Differentiating human from animal isolates of *Cryptosporidium parvum*. *Emerg Infect Dis.* **4**(4):681-5.
73. **Theodos, C. M.** 1998. Innate and cell-mediated immune responses to *Cryptosporidium parvum*. *Adv Parasitol.* **40**:87-119.
74. **Tzipori, S., and G. Widmer.** *in press.* The Biology of *Cryptosporidium*. *In F. Petry (ed.), Cryptosporidiosis and Microsporidiosis.* Karger.
75. **Ungar, B. L.** 1990. Enzyme-linked immunoassay for detection of *Cryptosporidium* antigens in fecal specimens. *J Clin Microbiol.* **28**(11):2491-5.

Principal Investigator: Last, first, middle: Khan, Wasif, Ali

76. **Ungar, B. L., R. H. Gilman, C. F. Lanata, and I. Perez-Schael.** 1988. Seroepidemiology of *Cryptosporidium* infection in two Latin American populations. *J Infect Dis.* **157**(3):551-6.
77. **Ungar, B. L., M. Mulligan, and T. B. Nutman.** 1989. Serologic evidence of *Cryptosporidium* infection in US volunteers before and during Peace Corps service in Africa. *Arch Intern Med.* **149**(4):894-7.
78. **Ungar, B. L., and T. E. Nash.** 1986. Quantification of specific antibody response to *Cryptosporidium* antigens by laser densitometry. *Infect Immun.* **53**(1):124-8.
79. **Ungar, B. L., R. Soave, R. Fayer, and T. E. Nash.** 1986. Enzyme immunoassay detection of immunoglobulin M and G antibodies to *Cryptosporidium* in immunocompetent and immunocompromised persons. *J Infect Dis.* **153**(3):570-8.
80. **Vasquez, J. R., L. Gooze, K. Kim, J. Gut, C. Petersen, and R. G. Nelson.** 1996. Potential antifolate resistance determinants and genotypic variation in the bifunctional dihydrofolate reductase-thymidylate synthase gene from human and bovine isolates of *Cryptosporidium parvum*. *Mol Biochem Parasitol.* **79**(2):153-65.
81. **Widmer, G.** 1998. Genetic heterogeneity and PCR detection of *Cryptosporidium parvum*. *Adv Parasitol.* **40**:223-39.
82. **Widmer, G., D. Akiyoshi, M. A. Buckholt, X. Feng, S. M. Rich, K. M. Deary, C. A. Bowman, P. Xu, Y. Wang, X. Wang, G. A. Buck, and S. Tzipori.** 2000. Animal propagation and genomic survey of a genotype 1 isolate of *Cryptosporidium parvum*. *Mol Biochem Parasitol.* **108**(2):187-197.
83. **Widmer, G., S. Tzipori, C. J. Fichtenbaum, and J. K. Griffiths.** 1998. Genotypic and phenotypic characterization of *Cryptosporidium parvum* isolates from people with AIDS. *J Infect Dis.* **178**(3):834-40.
84. **Xiao, L., U. M. Morgan, J. Limor, A. Escalante, M. Arrowood, W. Shulaw, R. C. Thompson, R. Fayer, and A. A. Lal.** 1999. Genetic diversity within *Cryptosporidium parvum* and related *Cryptosporidium* species. *Appl Environ Microbiol.* **65**(8):3386-91.
85. **Zu, S. X., J. F. Li, L. J. Barrett, R. Fayer, S. Y. Shu, J. F. McAuliffe, J. K. Roche, and R. L. Guerrant.** 1994. Seroepidemiologic study of *Cryptosporidium* infection in children from rural communities of Anhui, China and Fortaleza, Brazil. *Am J Trop Med Hyg.* **51**(1):1-10.
86. **Rahman M., Shahid N. S., Rahman H., Sack D. A., Rahman N., and Hossain S.** 1990. *Am J Trop Med Hyg.* **42**(2):127-30.

Dissemination and Use of Findings

Describe explicitly the plans for disseminating the accomplished results. Describe what type of publication is anticipated: working papers, internal (institutional) publication, international publications, international conferences and agencies, workshops etc. Mention if the project is linked to the Government of Bangladesh through a training programme.

The findings of the study will disseminated as follows:

1. Presentation(s) at scientific forums, ICDDR,B for dissemination among scientists of the Centre.
2. Presentation at the Annual Scientific Conference (ASCON) of ICDDR,B for dissemination amongst scientists and health officials of the Government of Bangladesh and of non-governmental organizations.
3. Presentation at regional and international scientific conferences.
4. Publication in peer-reviewed international medical journal.

Collaborative Arrangements

Describe briefly if this study involves any scientific, administrative, fiscal, or programmatic arrangements with other national or international organizations or individuals. Indicate the nature and extent of collaboration and include a letter of agreement between the applicant or his/her organization and the collaborating organization. (**DO NOT EXCEED ONE PAGE**)

This study is a part of ICIDR (International Collaborations in Infectious Disease Research) program at the NIH (National Institute of Health) that supports collaborations among PSG (Pathogen Specific Groups). The other two collaborative sites includes - Dr. Richard Guerrant's diarrhoea group in Brazil and Dr. Michael Bennish diarrhoea group in South Africa. Dr. Honorine Ward at NEMC (New England Medical Center) would look at *Cryptosporidium parvum* genotypes and anti-cryptosporidial stool and serum antibody responses in samples from all three ICIDR locations.

Biography of the Investigators

Give biographical data in the following table for key personnel including the Principal Investigator. Use a photocopy of this page for each investigator.

Name	Position	Date of Birth
1. Wasif Ali Khan	Assistant Scientist, CSD, ICDDR, B	19 January, 1963

Academic Qualifications (Begin with baccalaureate or other initial professional education)

Institution and Location	Degree	Year	Field of Study
1. Chittagong Medical College	M.B.B.S.	1987	Medicine & Surgery

Research and Professional Experience

Concluding with the present position, list, in chronological order, previous positions held, experience, and honours. Indicate current membership on any professional societies or public committees. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. (DO NOT EXCEED TWO PAGES, USE CONTINUATION SHEETS).

Bibliography

1. Khan WA, Begum M, Salam MA, Bardhan PK, MR, Mahalanabis D. Comparative trial of five antimicrobial compounds in the treatment of cholera in adults. *Trans Royal Soc Trop Med Hyg.* 1995;89:103-6.
2. Khan WA, Dhar U, Salam MA, Seas C. Bacterial meningitis in a diarrhoeal disease treatment centre in Bangladesh. *Acta Paediatr* 1995;84:693-4.
3. Khan WA, Dhar U, Begum M, Salam MA, Bardhan PK, Mahalanabis D. Antimicrobial treatment of cholera due to *Vibrio cholerae* O139 (Synonym Bengal). *Drugs (Extended abstract)* 1995;40 (Suppl.2):460-2.
4. Khan WA, Salam MA, Bennish ML. C reactive protein and prealbumin as markers of disease activity in shigellosis. *Gut* 1995;37:402-5.
5. Khan WA, Bennish ML, Seas C, Khan EH, Ronan A, Dhar U, Busch W, Salam MA. Randomised controlled comparison of single-dose ciprofloxacin and doxycycline for cholera caused by *Vibrio cholerae* O1 or O139. *Lancet* 1996;348:296-300.
6. Khan WA, Seas C, Dhar U, Salam MA, Bennish ML. Treatment of shigellosis: V. Comparison of azithromycin and ciprofloxacin. *Ann Intern Med* 1997;126:697-703.
7. Khan WA, Dhar U, Salam MA, Griffiths JK, Bennish ML. Central Nervous System Manifestations of Childhood Shigellosis: Prevalence, Risk Factors, and Outcome. *Pediatrics* 1999 103 (2): p. e18
<http://www.pediatrics.org/cgi/content/abstract/103/2/e18>
8. Kabir I, Khan WA, Haider R, Mitra AK, Alam AN. Erythromycin and trimethoprim-sulphamethoxazole in the treatment of cholera in children. *J Diarr Dis Res* 1996;14:243-7.

Principal Investigator: Last, first, middle: Khan, Wasif, Ali

Biography of the Investigators

Give biographical data in the following table for key personnel including the Principal Investigator. Use a photocopy of this page for each investigator.

Name	Position	Date of Birth
Honorine Ward	Assistant Professor	10/23/52

Academic Qualifications (Begin with baccalaureate or other initial professional education)

Institution and Location	Degree	Year	Field of study
1. University of Madras Christian Medical College, Vellore, India	MBBS (US equiv. MD)	1976	Medicine
2. Tufts University School of Medicine	Post-doctoral studies	1984-1988	Parasitology

Research and Professional Experience

Concluding with the present position, list, in chronological order, previous positions held, experience, and honours. Indicate current membership on any professional societies or public committees. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. (DO NOT EXCEED TWO PAGES, USE CONTINUATION SHEETS).

1975-1976 Intern,
Christian Medical College Hospital, Vellore, India

1976-1977 Junior Gastroenterology Fellow,
Wellcome Research Unit and Division of Gastroenterology,
Christian Medical College Hospital, Vellore, India

1977-1983 Gastroenterology Fellow,
Kothari Center of Gastroenterology,
The Calcutta Medical Research Institute, Calcutta

1984-1986 Postdoctoral Research Fellow,
Division of Geographic Medicine and Infectious Diseases,
New England Medical Center,
Tufts University School of Medicine, Boston, MA

1986-1988 Instructor,
Division of Geographic Medicine and Infectious Diseases,
New England Medical Center,
Tufts University School of Medicine, Boston, MA

1988- present Assistant Professor,
Division of Geographic Medicine and Infectious Diseases,
New England Medical Center,
Tufts University School of Medicine Boston, MA

1998- present Member, Immunology Program, Sackler School of Graduate Medical Sciences, Tufts
University School of Medicine Boston, MA

1999- present Assistant Professor
Tufts University School of Veterinary Medicine

AWARDS

1988 Maxwell Finland Award, Massachusetts Infectious Diseases Society

Bibliography

Principal Investigator: Last, first, middle: Khan, Wasif, Ali

1. Lev, B., Ward, H., Keusch, G.T., and Pereira M. E. A. Lectin activation in *Giardia lamblia* by host protease: A novel host parasite interaction. *Science* 232, 71-73 1986
2. Hemell, O., Ward, H., Blackberg, L., and Pereira, M. E. A. Killing of *Giardia lamblia* by human milk lipases: an effect mediated by lipolysis of milk lipids *Journal of Infectious Diseases* 153, 715-720 1986
3. Ward, H. D., Lev, B. I., Kane, A. V., Keusch G. T., Pereira, M. E. A. Identification and characterization of taglin: a trypsin activated mannose-6-phosphate binding lectin from *Giardia lamblia* *Biochemistry* 26,8669-8675 1987
4. Ward, H. D., Lev, B. I., Keusch G. T., Pereira, M. E. A. Induction of lectin activity in *Giardia* In UCLA Symposium, Molecular strategies of parasite invasion Alan R. Liss, Inc., 521-530 1987
5. Ward, H. D., Alroy, J., Lev, B. I., Keusch G. T., Pereira, M. E. A. Biology of *Giardia lamblia* : Detection of *N*-Acetyl-D-glucosamine as the only surface saccharide moiety and identification of two distinct subsets of trophozoites by lectin binding. *Journal of Experimental Medicine* 167, 73-88 1988
6. Alroy, J., Goyal, V., Lukacs, N. W., Taylor, R. L., Strout, R. G., Ward, H. D. and Pereira, M. E. A. Glycoconjugates of intestinal epithelium of the domestic fowl (*Gallus domesticus*): A lectin histochemistry study *Histochemical Journal* 21, 187-193 1989
7. Ward, H. D., Keusch G. T., Pereira, M. E. A. Induction of a phosphomannosyl binding activity in *Giardia*. *Bioessays* 12, 211-215 1990
8. Ortega-Barria, E. Ward, H. D., Evans, J., Pereira, M. E. A. N-acetyl-D-glucosamine is present in cysts and trophozoites of *Giardia lamblia* and serves as receptor for wheatgerm agglutinin. *Molecular and Biochemical Parasitology*43:151-165 1990
9. Ward, H.D., Kane, A.V., Ortega-Barria, E., Keusch, G.T., and Pereira, M.E.A. Identification of developmentally regulated *Giardia lamblia* cyst antigens using GCSA-1 a cyst specific monoclonal antibody. *Molecular Microbiology* 12, 2095-2102 1990
10. Kane, A.V., Ward, H.D., Keusch, G.T. and Pereira, M.E.A. *In Vitro* encystation of *Giardia lamblia*: large scale production of *in vitro* cysts and strain and clone differences in encystation efficiency. *Journal of Parasitology* 77, 974-981 1991
11. Reiner, D. S. Hetsko, M., Das, S., Ward, H.D., McCaffrey, M. and Gillin, F. D. *Giardia lamblia*: Defective cyst antigen expression, secretory vesicle formation, and bile salt transport in an encystation deficient subline. *Experimental Parasitology* 77, 461-72 1993
12. Hamer, D. H., Ward, H. D., Tzipori, S., Pereira, M. E. A., Alroy, J. and Keusch G. T. Attachment of *Cryptosporidium parvum* sporozoites to MDCK cells in vitro. *Infection and Immunity* 1994 62; 2208-2213
13. Ortega-Barria, E. Ward, H. D., Keusch, G. T. and Pereira, M. E. A. Growth inhibition by a dietary lectin is associated with arrest of the cell cycle *Journal of Clinical Investigation* 94, 2283-2288 1994
14. A. Joe, D.H. Hamer, M. Kelley, M.E.A. Pereira, G.T. Keusch, S. Tzipori, and H.D. Ward. Role of a Gal/GalNAc specific sporozoite surface lectin in *C. parvum*-host cell interaction. *Journal of Eukaryotic Microbiology* 41, 44S 1994
15. Strout, R. G., Alroy, J., Lukacs, N. W., Ward, H.D., Pereira, M.E.A. Developmentally regulated lectins in *Eimeria* species and their role in avian coccidiosis *Journal of Parasitology*, 80, 946-951 1994
16. Keusch, G. T., Hamer, D., Joe, A., Kelly, M., Griffiths, J. Ward, H. D. *Cryptosporidia*- who is at risk? *Schweiz Med Wochenschr* 125, 899-908 1995
17. Ward, H. D. Glycobiology of parasite infection In H.-J. Gabius, and S. Gabius (ed.), *Glycosciences: Status and Perspectives*. Chapman & Hall GmbH, Weinheim 1996
18. Verdon, R., Keusch, G. T., Tzipori, S., Grubman, S. M., Jefferson D. M. and Ward, H. D. An in vitro model of infection of human biliary epithelial cells by *Cryptosporidium parvum*. *Journal of Infectious Diseases* 175:1268-72 1997
19. Ward, H. D. and Cevallos, A.M. *Cryptosporidium*: Molecular basis of Host-Parasite Interaction *Advances in Parasitology* 40:151-185, 1998
20. Joe, A., Verdon, R. Keusch, G.T., Tzipori, S., and Ward H.D. Attachment of *Cryptosporidium parvum* sporozoites to human intestinal epithelial cells *Infection and Immunity*, 66:3429-3432 1998
21. Barnes, D. A., Bonnin, A., Huang, J-X. Gousset, L., Wu, J., Gut, J., Doyle, P., Dubremetz, J-F., Ward, H., Petersen, C. A novel multi-domain mucin-like glycoprotein of *Cryptosporidium parvum* mediates invasion. *Molecular and Biochemical Parasitology*, 96: 93-110, 1998
22. Baecher-Allan CM, Santora K, Sarantopoulos S, Den W, Sompuram SR, Cevallos AM, Bhat N, Ward H. Sharon J, Generation of a polyclonal Fab phage display library to the protozoan parasite *Cryptosporidium parvum* *Combinatorial Chemistry and High Throughput Screening* 1999 2:319-25
23. Bhat, N. and Ward, H.D. Lectin-mediated Interaction of Parasites with Host cells in Lectins and Pathology, Eds M.Caron and A.P. Seve, Harwood Academic Publishers, Amsterdam, 173-200, 2000
24. Cevallos A. M, Zhang X, Waldor M, Jaison S, Tzipori S, Zhou X, Neutra M, Ward H. D. Molecular cloning and expression of a gene encoding *Cryptosporidium parvum* glycoproteins gp40 and gp15. *Infection and Immunity* 68:4108-4116 2000
25. Cevallos, A. M., Verdon, R., Hamer, D., Stein, B., Tzipori, S., Pereira, M. E. A., Keusch, G. T. and Ward, H. D. Mediation of *Cryptosporidium parvum* infection in vitro by mucin-like glycoproteins defined by a neutralizing monoclonal antibody *Infection and Immunity* 2000 (in press)
26. Joe, A., Bhat, N., Keusch, G.T., Tzipori, S., Pereira, M.E.A. and Ward H. D. Purification and characterization of a Gal/GalNAc specific sporozoite surface lectin of *Cryptosporidium parvum* (submitted)
27. Bhat, N., Bhaskar, K. R. Tzipori S., and Ward, H. D. Interaction of *Cryptosporidium parvum* sporozoites with intestinal mucins (submitted)

Principal Investigator: Last, first, middle: Khan, Wasif, Ali

Biography of the Investigators

Give biographical data in the following table for key personnel including the Principal Investigator. Use a photocopy of this page for each investigator.

Name	Position	Date of Birth
Stephen B. Calderwood	Professor, Harvard Medical School	4/9/46

Academic Qualifications (Begin with baccalaureate or other initial professional education)

Institution and Location	Degree	Year	Field of Study
1. Harvard University, Cambridge MA	A.B.	1971	Biology
2. Harvard Medical School	M.D.	1975	Medicine

Research and Professional Experience

Concluding with the present position, list, in chronological order, previous positions held, experience, and honours. Indicate current membership on any professional societies or public committees. List, in, chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. (DO NOT EXCEED TWO PAGES, USE CONTINUATION SHEETS).

Research and Professional Experience

- 1975-78 Medical Residency, Massachusetts General Hospital, Boston, MA
- 1978-80 Clinical and Research Fellow in Infectious Diseases, Massachusetts General Hospital
- 1981 Chief Resident in Medicine, Massachusetts General Hospital
- 1981-84 Instructor in Medicine, Harvard Medical School, Boston, MA
- 1984-88 Assistant Professor of Medicine, Harvard Medical School
- 1985-88 Upjohn Scholar, Department of Microbiology and Molecular Genetics (Laboratory of Dr. John Mekalanos), Harvard Medical School
- 1988-91 Assistant Professor of Medicine (Microbio. & Molecular Genetics), Harvard Medical School
- 1991- Associate Professor of Medicine (Microbio. & Molecular Genetics), Harvard Medical School
- 1992- Editorial Board, *Infection and Immunity*
- 1994-98 Bacteriology and Mycology-1 Study Section, National Institutes of Health, Bethesda, MD
- 1995- Chief, Division of Infectious Diseases, Massachusetts General Hospital
- 1996-98 Chair, Bact. & Mycology-1 Study Section, National Institutes of Health, Bethesda, MD
- 2000- Professor of Medicine (Microbiology and Molecular Genetics), Harvard Medical School

Awards and Honors

- 1971 Summa cum laude
- 1971 Phi Beta Kappa
- 1975 Alpha Omega Alpha

Selected Original Publications (out of a total of 67):

1. Goldberg MB, Boyko SA, Calderwood SB. Positive transcriptional regulation of an iron-regulated virulence gene in *Vibrio cholerae*. Proc Natl Acad Sci USA 1991; 88:1125-1129.
2. Litwin CM, Calderwood SB. Analysis of the complexity of regulation by Fur in *Vibrio cholerae*. J Bacteriol 1994; 176:240-248.
3. Lam MS, Litwin CM, Carroll PA, Calderwood SB. *Vibrio cholerae fur* mutations associated with loss of repressor activity: Implications for the structural-functional relationships of Fur. J Bacteriol 1994; 176:5108-5115.
4. Butterson JR, Calderwood SB. Identification, cloning and sequencing of a gene required for ferric vibriobactin utilization by *Vibrio cholerae*. J Bacteriol 1994; 176:5631-5638.
5. Butterson JR, Beattie DT, Gardel CG, Carroll PA, Hyman T, Killeen KP, Mekalanos JJ, Calderwood SB. Heterologous antigen expression in *Vibrio cholerae* vector strains. Infect Immun 1995; 63:2689-2696.
6. Tashima KT, Carroll PA, Rogers MB, Calderwood SB. Relative importance of three iron-regulated outer membrane proteins for *in vivo* growth of *Vibrio cholerae*. Infect Immun 1996; 64:1756-1761.

Principal Investigator: Last, first, middle: Khan, Wasif, Ali

7. Butterson JR, Shahin RA, Calderwood SB. Development of a germ-free mouse model of *Vibrio cholerae* infection. *Infect Immun* 1996; 64:4373-4377.
8. Carroll PA, Zhao G, Boyko SA, Winkler ME, Calderwood SB. Identification, sequencing, and enzymatic activity of the erythrose 4-phosphate dehydrogenase gene of *Vibrio cholerae*. *J Bacteriol* 1997; 179:293-296.
9. Watnick PI, Eto T, Takahashi H, Calderwood SB. Purification of *Vibrio cholerae* Fur and estimation of its intracellular concentration by antibody sandwich enzyme-linked immunosorbent assay. *J Bacteriol* 1997; 179:243-247.
10. Butterson JR, Ryan ET, Acheson DWK, Calderwood SB. Co-expression of the B subunit of Shiga toxin 1 and EaeA from enterohemorrhagic *Escherichia coli* in *Vibrio cholerae* vaccine strains. *Infect Immun* 1997; 65:2127-2135.
11. Ryan ET, Butterson JR, Smith RN, Carroll PA, Crean TI, Calderwood SB. Protective immunity against *Clostridium difficile* toxin A induced by oral immunization with a live, attenuated *Vibrio cholerae* vector strain. *Infect Immun* 1997; 65:2941-2949.
13. Carroll PA, Tashima KT, Rogers MB, DiRita VJ, Calderwood SB. Phase variation in *tcpH* modulates expression of the ToxR regulon in *Vibrio cholerae*. *Mol Microbiol* 1997; 25:1099-1111.
14. Rahme LG, Tan M-W, Le L, Wong SM, Tompkins RG, Calderwood SB, Ausubel FM. Use of model plant hosts to identify *Pseudomonas aeruginosa* virulence factors. *Proc Natl Acad Sci USA* 1997; 94:13245-13250.
15. Pegues DA, Colby C, Hibberd PL, Cohen LG, Ausubel FM, Calderwood SB, Hooper DC. The epidemiology of resistance to ofloxacin and oxacillin among clinical coagulase-negative staphylococcal isolates: analysis of risk factors and strain types. *Clin Infect Dis* 1998; 26:72-79.
16. Calia KE, Waldor MK, Calderwood SB. Use of representational difference analysis to identify genomic differences between pathogenic strains of *Vibrio cholerae*. *Infect Immun* 1998; 66:849-852.
17. Watnick PI, Butterson JR, Calderwood SB. The interaction of the *Vibrio cholerae* transcription factors, Fur and IrgB, with the overlapping promoters of two virulence genes, *irgA* and *irgB*. *Gene* 1998;209:65-70
18. John MDV, Hibberd PL, Karchmer AW, Sleeper LA, Calderwood SB. *Staphylococcus aureus* prosthetic valve endocarditis: Optimal management and risk factors for death. *Clin Infect Dis* 1998;26:1302-1309.
19. Mylonakis E, Hohmann EL, Calderwood SB. Central nervous system infection with *Listeria monocytogenes*: Analysis of 820 episodes from a 33 year experience at one hospital and review of the literature. *Medicine* 1998; 77:313-337.
20. Wong SM, Carroll PA, Rahme LG, Ausubel FM, Calderwood SB. Modulation of expression of the ToxR regulon in *Vibrio cholerae* by a member of the two-component family of response regulators. *Infect Immun* 1998; 66:5854-5861.
21. Lin W, Fullner KJ, Clayton R, Sexton JA, Rogers MB, Calia KE, Calderwood SB, Fraser C, Mekalanos JJ. Identification of a *Vibrio cholerae* RTX toxin gene cluster that is tightly linked to the cholera toxin prophage. *Proc Natl Acad Sci USA* 1999; 96:1071-1076.
22. Ryan ET, Crean TI, John M, Butterson JR, Clements JD, Calderwood SB. In vivo expression and immunoadjuvancy of a mutant of heat-labile enterotoxin of *Escherichia coli* in vaccine and vector strains of *Vibrio cholerae*. *Infect Immun* 1999; 67:1694-1701.
23. Murley YM, Carroll PA, Skorupski K, Taylor RK, Calderwood SB. Differential transcription of the *tcpPH* operon confers biotype-specific control of the *Vibrio cholerae* ToxR virulence regulon. *Infect Immun* 1999; 67:5117-5123.
24. Konadu E, Donohue-Rolfe A, Calderwood SB, Poszgay V, Shiloach J, Robbins JB, Szu SC. *Escherichia coli* O157-O-specific polysaccharide and B subunit Shiga toxin 1 conjugates: synthesis and immunologic properties in mice. *Infect Immun* 1999; 67:6191-6193.
25. Ryan ET, Crean TI, Kochi SK, John M, Luciano A, Killeen KP, Klose KE, Calderwood SB. Development of a *ΔglnA* balanced lethal plasmid system for expression of heterologous antigens by attenuated vaccine vector strains of *Vibrio cholerae*. *Infect Immun* 2000; 68:221-226.
26. John M, Crean TI, Calderwood SB, Ryan ET. In vitro and in vivo analyses of constitutive and in-vivo induced promoters in attenuated vaccine and vector strains of *Vibrio cholerae*. *Infect Immun* 2000; 68:1171-1175.
27. Butterson JR, Choi MH, Watnick PI, Carroll PA, Calderwood SB. *Vibrio cholerae* VibF is required for vibriobactin synthesis and is a member of the family of nonribosomal peptide synthetases. *J Bacteriol* 2000; 182:1731-1738.
28. Murley YM, Behari J, Griffin R, Calderwood JB. Classical and El Tor biotypes of *Vibrio cholerae* differ in timing of transcription of *tcpPH* during growth in inducing conditions. *Infect Immun* 2000; 68:3010-3014.
29. Rogers MB, Sexton JA, DeCastro GJ, Calderwood SB. Identification of an operon required for ferrichrome-iron utilization in *Vibrio cholerae*. *J Bacteriol* 2000; 182:2350-2353.

Biography of the Investigators

Give biographical data in the following table for key personnel including the Principal Investigator. Use a photocopy of this page for each investigator.

Name	Position	Date of Birth
Edward T. Ryan	Instructor, Harvard Medical School	09/05/62

Academic Qualifications (Begin with baccalaureate or other initial professional education)

Institution and Location	Degree	Year	Field of Study
1. Princeton University, Princeton, NJ	A.B.	1984	Biochemical Sciences
2. Harvard University, Boston, MA	M.D.	1988	Medicine
3. London Schl. Hyg & Trop Med, London	DTM&H	1994	Tropical Medicine

Research and Professional Experience

Concluding with the present position, list, in chronological order, previous positions held, experience, and honours. Indicate current membership on any professional societies or public committees. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. (DO NOT EXCEED TWO PAGES, USE CONTINUATION SHEETS).

Research and Professional Experience

- 1988-91 Intern, Junior Resident and Senior Resident, Internal Medicine, Massachusetts General Hospital, Boston, MA
- 1991-92 Clinical Fellow, Infectious Disease Unit; Massachusetts General Hospital, Boston, MA
- 1992 Physician, Department of Internal Medicine, FHP-Asia; Guam Memorial Hospital, Guam
- 1993 Chief Resident in Medicine, Massachusetts General Hospital, Boston, MA
- 1993- Instructor in Medicine, Harvard Medical School, Boston, MA
- 1994 Dalton Traveling Fellow, London School of Hygiene and Tropical Medicine, London, UK
- 1994-97 Clinical and Research Fellow, Infectious Disease Unit, Massachusetts General Hospital, Boston, MA
- 1996-97 Fellow in Human Rights & Medicine; Center for the Study of Society & Medicine, College of Physicians & Surgeons, Columbia University, New York, NY
- 1996-97 Warren-Whitman-Richardson Traveling Fellow, Harvard Medical School, Boston, MA
- 1997 Visiting Scientist/International Fellow, International Centre for Diarrhoeal Disease Research (ICDDR,B), Dhaka, Bangladesh
- 1997 Assistant in Medicine, Massachusetts General Hospital, Boston, MA
- 1998 Assistant in Pediatrics, Massachusetts General Hospital, Boston, MA
- 1998 Director, Travel Advice & Immunization Center, Massachusetts Gen. Hospital, Boston, MA
- 1998 Director, Tropical & Geographic Medicine Center, Massachusetts Gen. Hospital, Boston, MA
- 2000 Assistant Physician, Massachusetts General Hospital

Awards and Honors

- 1984 *Summa Cum Laude* in Biochemical Sciences, Princeton University
- 1984 Phi Beta Kappa, Princeton University
- 1988 *Cum Laude et Thesi Propria*, Harvard University
- 1994 Duncan Gold Medal, First Prize, London School of Hygiene and Tropical Medicine
- 1994 Edward H. Kass Award for Clinical Excellence, Massachusetts Infectious Diseases Society

Bibliography

Articles

1. Marti GE, Ryan ET., Papadopoulos NM, Filling-Katz M, Barton N, Fleischer TA, Rick M, Gralnick HR. Polyclonal B-cell lymphocytosis and hypergammaglobulinemia in patients with Gaucher's disease. *Am J Hematol* 1988;29:188-194.
2. Ryan ET. Surgical nosocomial infections. *Res S Phys* 1991;2:11-15.

Principal Investigator: Last, first, middle: Khan, Wasif, Ali

3. Ryan ET, Ecker JL, Christakis NA, Folkman J. Hirschsprung's disease: associated abnormalities and demography. *J Ped Surg* 1992;27:76-81.
4. Ryan ET, Pak PH, DeSanctis RW. Myocardial infarction mimicked by acute cholecystitis. *Ann Intern Med* 1992;116:218-220.
5. Young JA, Ryan ET. Parasitic infections of the anterior segment. *International Ophthalmol. Clinics* 1996;36:49-71.
6. Butterson JR, Ryan ET, Shahin RA, Calderwood SB. Development of a germfree mouse model of *Vibrio cholerae* infection. *Infect. Immun.* 1996;64:4373-4377.
7. Butterson JR, Ryan ET, Acheson DWK, Calderwood SB. Coexpression of the B subunit of Shiga toxin 1 and EaeA from enterohemorrhagic *Escherichia coli* in *Vibrio cholerae* vaccine strains. *Infect. Immun.* 1997;65:2127-2135.
8. Ryan ET, Butterson JR, Smith RN, Carroll PA, Crean TI, Calderwood SB. Protective immunity against *Clostridium difficile* toxin A induced by oral immunization with a live, attenuated *Vibrio cholerae* vector strain. *Infect. Immun.* 1997;65:2941-2949.
9. Ryan ET, Butterson JR, Zhang T, Baker MA, Stanley Jr SL, Calderwood SB. Oral immunization with attenuated vaccine strains of *Vibrio cholerae* expressing a dodecapeptide repeat of the serine rich *Entamoeba histolytica* protein (SREHP-12) fused to the cholera toxin B subunit induces systemic and mucosal antiamebic and anti-*V. cholerae* antibody responses in mice. *Infect. Immun.* 1997;65:3118-3125.
10. Ryan ET, Crean TI, John M, Butterson JR, Clements JD, Calderwood SB. In vivo expression and immunoadjuvancy of a mutant of heat-labile enterotoxin of *Escherichia coli* in vaccine and vector strains of *Vibrio cholerae*. *Infect. Immun.* 1999;67:1694-1701.
11. Ryan ET, Crean TI, Kochi SK, John M, Luciano AA, Calderwood SB. Development of a $\Delta glnA$ balanced lethal plasmid system for expression of heterologous antigens by attenuated vaccine vector strains of *Vibrio cholerae*. *Infect. Immun.* 2000;68:221-226.
12. Crean TI, John M, Calderwood SB, Ryan ET. Optimizing the germ-free mouse model for in vivo evaluation of oral *Vibrio cholerae* vaccine and vector strains. *Infect. Immun.* 2000;68:977-981.
13. John M, Crean TI, Calderwood SB, Ryan ET. In vitro and in vivo analyses of constitutive and in vivo-induced promoters in attenuated vaccine and vector strains of *Vibrio cholerae*. *Infect. Immun.* 2000;68:1171-1175.
14. Ryan ET, Dhar U, Khan WA, Salam MA, Faruque ASG, Fuchs GJ, Calderwood SB, Bennis ML. Mortality, morbidity and microbiology of endemic cholera in Dhaka, Bangladesh. *Am. J. Trop. Med. Hygiene* (in press).

Texts/Reviews

1. Hibberd PL, Ryan ET, Baker AS. *Pneumocystis carinii*, parasitic infection, and the eye. In: Albert DM, Jakobiec FA, eds. *Principles and Practice of Ophthalmology*. Philadelphia: WB Saunders, 1994:3065-3077.
2. Ryan ET, Baker AS. Systemic bacterial infections and the eye. In: Albert DM, Jakobiec FA, eds. *Principles and Practice of Ophthalmology*. Philadelphia: WB Saunders, 1994:3006-3010.
3. Ryan ET, Swartz MN. Dermatological Infections. In: Roitt IM, Delves PJ, eds. *Encyclopedia of Immunology*, Second Edition. London. Academic Press Ltd., 1998:750-754.
4. Ryan ET, Maguire JH. Parasitic infections and the eye. In: Albert DM, Jakobiec FA, eds. *Principles and Practice of Ophthalmology*. Philadelphia: WB Saunders, 2000:4917-4939.
5. Ryan ET. Ocular disease. In: Guerrant RL, Walker DH, Weller PF, eds. *Tropical Infectious Diseases: Principles, Pathogens and Practice*. Philadelphia: Churchill Livingstone, 1999:1485-1541.
6. Ryan ET. Tropical and travel medicine: literature alert. *Current Infectious Disease Reports* 1999;1(5):411-412.
7. Ryan ET. Tropical and travel medicine: literature alert. *Current Infectious Disease Reports* 2000;1(2):3-4.
8. Ryan ET. Immunization. In: Gorroll AH, May LA, Mulley Jr AG, eds. *Primary Care Medicine: Office Evaluation and Management of the Adult Patient*. Philadelphia: JB Lippincott Co.; (in press).
9. Ryan ET, Calderwood SB. Cholera vaccines. *J Travel Med* (in press).
10. Ryan ET, Kain KC. Health advice and immunizations for travelers. *N Engl J Med* 2000;342:1716-1725.
11. Ryan ET. Ocular disease. In: Guerrant RL, Walker DH, Weller PF, eds. *Essentials of Tropical Infectious Diseases*. Philadelphia: Churchill Livingstone (in press).
12. Ryan ET. Malaria: epidemiology, pathogenesis, diagnosis, prevention and treatment: an update. In: Remington JS, Swartz MN, eds. *Current Clinical Topics in Infectious Diseases*. Boston: Blackwell Science (in press).
13. Ryan ET, Calderwood SB. Cholera vaccines. *Clin Infect Dis* (in press).

Detailed Budget for New Proposal

Project Title: MOLECULAR EPIDEMIOLOGY AND HUMORAL IMMUNE RESPONSES TO *CRYPTOSPORIDIUM PARVUM*

Name of PI: Wasif Ali Khan

Protocol Number: **Name of Division:** Clinical Sciences Division (CSD)

Funding Source: NIH

Amount Funded (direct): US\$ 10,512

Overhead (31%): US\$ Nil

Total: US\$ 10,512

Starting Date: ASAP

Closing Date: One year from recruitment of first study patient

Strategic Plan Priority Code(s): 11

Sl. No	Account Description	Salary Support			US \$ Amount Requested
		Position	Effort %	Salary (8 months)	Total
1	Wasif Ali Khan	NO-B	30	220 x 8	1760
2	Research Officer	GS-5	20	55 x 8	440
3	Lab. Technician	GS-3	10	17.3 x 8	138
4	Ward Attendant (2)	GS-2	25	56 x 8 x 2	576
Sub Total					2,914

Supplies and Materials (Description of Items)		US \$ Amount Requested
1	Office supplies	400
2	Lab. Supplies	200
3	Hospital supplies	200
Sub Totals		800

Interdepartmental Services		Total
1	Pathological Tests (stool microscopy for cryptosporidium - 2550 x @\$2.47)	6,298
2	Microbiological, Biochemistry tests	300
3	Medical illustration	100
4	Xerox, Mimeographs etc.	100
Subtotal		6,798

Total Direct Cost:	\$ 10,512
Overhead Cost (31% of Direct):	\$ Nil
TOTAL PROJECT COST:	\$10,512

S. Moir 18/2000
Shamima Moir
Controller, Budget & Costing

Budget Justifications

Please provide one page statement justifying the budgeted amount for each major item. Justify use of man power, major equipment, and laboratory services.

1. Personnel

This study will examine an estimated 2500 (isolation rate of cryptosporidium is 2%) stool samples from children ≤ 5 years of age coming with persistent diarrhoea, fulfilling the study eligibility criteria to get 50 evaluable patients over 8 month period. An equal number of age matched children ($n=50$) with diarrhoea but not infected with *Cryptosporidium parvum* will also be enrolled. A questionnaire will be completed by the investigator who will be assisted by the research officer. The clinical care for these patients will be provided by the investigators of this study.

In addition, the research officer will be responsible for ensuring that the laboratory investigations of the study are done on time and to compile and maintain results of all laboratory tests. The research officer will also be responsible for appropriately processing, labelling and storing blood and stool samples.

Ward attendants will be required to initially identify potential study patients. They will carry the samples to the laboratory and provide the results to the investigator. Since patient recruitment will be done on all days, it will be essential to recruit two ward attendants.

2. Supply and material

These reflect exact cost of supplies and materials, which will be required for the study.

Principal Investigator: Last, first, middle: Khan, Wasif, Ali

Other Support

Describe sources, amount, duration, and grant number of all other research funding currently granted to PI or under consideration. (DO NOT EXCEED ONE PAGE FOR EACH INVESTIGATOR)

1. One study, short title "Short-course ciprofloxacin therapy for childhood shigellosis due to *S. dysenteriae* type I" is being conducted. The study is sponsored by New England Medical Center, Boston, MA. and has a budget of about US \$ 100,000.
2. Another protocol, short title " Azithromycin single dose therapy in childhood cholera" is being conducted. The study is sponsored by New England Medical Center, Boston, MA. and has a budget of US \$ 160,594.
3. Research protocol, short titled "Diagnosis of pneumonia in children with dehydrating diarrhoea". This study is currently undergoing, funded by USAID under the competitive grant agreement (tentative budget = US \$ 50,000)

Principal Investigator: Last, first, middle: Khan, Wasif, Ali

Other Support

Describe sources, amount, duration, and grant number of all other research funding currently granted to PI or under consideration. (DO NOT EXCEED ONE PAGE FOR EACH INVESTIGATOR)

Honorine Ward

NIH-NIAID

Evaluation of Anti-*C. parvum* polyclonal antibody libraries

9/1/96-5/31/01

\$ 138,882 total direct costs/current year

American Water Works Association Research Foundation

Structural Physiology of the *Cryptosporidium* Oocyst Wall as it Relates to Drinking Water Treatment

01/01/00-6/30/02

\$ 173,915 total direct costs/current year

NIH-NIAID

Micronutrients and Enteric Infection in African Children

09/01/99-8/31/04

\$ 244,097 total direct costs/current year

USDA

Role of *Cryptosporidium parvum* surface glycoproteins in Host-Parasite Interactions

011/01/00-10/31/03

\$ 84,034 total direct costs/current year

Ethical Assurance for Protection of Human Rights

Describe in the space provided the justifications for conducting this research in human subjects. If the study needs observations on sick individuals, provide sufficient reasons for using them. Indicate how subject's rights are protected and if there is any benefit or risk to each subject of the study.

Justifications for conducting this research in human subjects

The objective of this study is to determine the genotypes of isolates from children with *C. parvum* infection and correlate with geographic location, age, clinical status (duration and severity of illness), drinking water source and contact with animal.

Protection of human rights

The intent of the research program, the study protocol, and the informed consent form to be used in the study will be submitted to the Ethical Review Committee of ICDDR, B. The study will only be initiated only after approval by the Committee.

Informed consent

Informed consent must be obtained from their guardian in accordance with the Declaration of Helsinki. It will be the responsibility of the investigator to obtain written informed consent from them after adequate explanation of the aims, methods, anticipated benefits and potential hazards of the study. The investigator must also explain to the guardian that they are completely free to refuse to enter in the study, and also to withdraw them self from the study at any time. This informed consent must be obtained from the guardian in the presence of a witness along with their signature and date.

Check List

After completing the protocol, please check that the following selected items have been included.

- | | |
|--|-------------------------------------|
| 1. Face Sheet Included | <input checked="" type="checkbox"/> |
| 2. Approval of the Division Director on Face Sheet | <input checked="" type="checkbox"/> |
| 3. Certification and Signature of PI on Face Sheet, #9 and #10 | <input checked="" type="checkbox"/> |
| 4. Table on Contents | <input checked="" type="checkbox"/> |
| 5. Project Summary | <input checked="" type="checkbox"/> |
| 6. Literature Cited | <input checked="" type="checkbox"/> |
| 7. Biography of Investigators | <input checked="" type="checkbox"/> |
| 8. Ethical Assurance | <input checked="" type="checkbox"/> |
| 9. Consent Forms | <input checked="" type="checkbox"/> |
| 10. Detailed Budget | <input checked="" type="checkbox"/> |

APPENDIX-1

International Centre for Diarrhoeal Disease Research, Bangladesh

Voluntary Consent Form

Title of the Research Project: MOLECULAR EPIDEMIOLOGY OF CRYPTOSPORODIOSIS

Principal Investigator: Wasif Ali Khan

Before recruiting into the study, the study subject must be informed about the objectives, procedures, and potential benefits and risks involved in the study. Details of all procedures must be provided including their risks, utility, duration, frequencies, and severity. All questions of the subject must be answered to his/ her satisfaction, indicating that the participation is purely voluntary. For children, consents must be obtained from their parents or legal guardians. The subject must indicate his/ her acceptance of participation by signing or thumb printing on this form.

Your child is suffering from persistent diarrhoea that we suspect might be due to *Cryptosporidium parvum* infection. This is an intestinal bug about which we know very little. Cryptosporidial infection in early childhood may result in subsequent impairment in growth, physical fitness and intellectual functions. We are conducting a study to determine the incidence of different types of cryptosporidial infections and correlate these types with clinical findings and immune responses. This will help us understand this infection better, and may allow us to develop ways of controlling, treating and preventing this infection. At present, there is no optimal treatment available for individuals with cryptosporidial infections; most children clear the infection on their own but some children are unable to clear the infection and the chronic diarrhea results. We would like your child to participate in the study.

If you agree to participate, your child will receive the usual care that any patient receives in this hospital. The only additional testing that will be done is that we will obtain a single blood and a single stool sample that we would not normally obtain. We will examine the stool to see if your child is infected with *Cryptosporidium parvum*, and we will measure immune responses in the stool and in your child's blood.

If you agree to have your child participate in this study, you can expect the following:

1. A detailed medical history and physical examination will be performed and noted on to a case report form (CRF).
2. Stool specimens will be collected from your child once.
3. Blood will be drawn from your child only once 1ml (1/5 of teaspoonfuls) from the elbow vein. This is a very small amount of blood, and it is safe to obtain this amount of blood from children.
4. We will keep all your child's medical information and the results of laboratory tests confidential; no one other than the investigators of this study, appropriately designated monitors from the U.S. National Institutes of Health (who are overseeing and funding this study) and members of the Ethical Review Committee of this Centre can have an access to this information after discharge from the hospital. All records will be locked in a safe cabinet. If you are interested to know the results of the laboratory tests performed we will be happy to provide that information to you when they become available; we would, however, like to inform you that results of many of these tests may become available long after the study is over.
5. You are the only person to decide whether or not your child will participate in this study. Your child will receive the excellent standard of care and treatment at this hospital even if you do not allow your child to participate in the study, and even if you withdraw your consent to participate at any time during the study.

Principal Investigator: Last, first, middle: Khan, Wasif, Ali

If you agree for participation in the study, please put your signature, or your left thumb impression at the specified space below. Thank you for your cooperation.

Signature
Patient / LTI of the guardian

Date

Signature of Investigator

Date

Witness's signature

Date

The Name, Designation and Contact Telephone Number of the Investigator:

Dr. Wasif Ali Khan
Assistant Scientist
Cholera Hospital
Mohakhali, Dhaka 1212
Direct: 9886734

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র, বাংলাদেশ।

ক্রিপটোস্পিরিয়ডসিস রোগের অনুবৈজ্ঞানিক প্রাদুর্ভাব।

সম্মতি পত্র

আপনার শিশু দীর্ঘ মেয়াদী ডায়রিয়াতে ভুগছে। আমরা ধারণা করছি যে এই ডায়রিয়া ক্রিপটোস্পিরিডিয়াম পারভাম নামক একটি জীবাণু দ্বারা বাহিত হয়েছে। ইহা একটি অশ্রবাহী জীবাণু, যাহা সম্পর্কে আমাদের জ্ঞান অত্যন্ত সীমিত। ক্রিপটোস্পিরিয়ডসিস রোগের কারণে শিশুদের দৈহিক, মানসিক ও মেধা বিকাশ বাধাগ্রস্ত হতে পারে। আমরা একটি গবেষণা করছি যাতে করে বিভিন্ন প্রকারের ক্রিপটোস্পিরিয়ডসিস রোগের ধরন এবং এই রোগের বিভিন্ন উপসর্গ এবং মানব শরীরে এর রোগ প্রতিরোধ ক্ষমতা সম্পর্কে জানা যাবে। এই গবেষণা আমাদের এই রোগ সংক্রমন সম্পর্কে জানতে অধিকতর সাহায্য করবে এবং এই রোগের নিয়ন্ত্রণ, চিকিৎসা, প্রতিরোধ কল্পে প্রয়োজনীয় ব্যবস্থা গ্রহণে সহায়তা করবে। বর্তমানে এই রোগের কোন সুনির্দিষ্ট চিকিৎসা নেই। অধিকাংশ শিশু বিনা চিকিৎসাতেই সুস্থ হয়ে যায়, কিছু ক্ষেত্রে এই রোগ দীর্ঘ মেয়াদী ডায়রিয়াতে পরিনত হয়।

আমরা আপনার শিশুকে এই গবেষণায় অংশগ্রহণের জন্য অনুরোধ করছি।

এই গবেষণায় অংশগ্রহণ করলে আপনার শিশু এই হাসপাতালের প্রচলিত সকল প্রকার চিকিৎসা পাবে। এছাড়া মাত্র একবার রক্ত ও মল পরীক্ষা করা হবে যা সাধারণত অন্যান্য রোগীর ক্ষেত্রে করা হয় না। আপনার শিশুর মলে ক্রিপটোস্পিরিডিয়াম পারভাম জীবাণু আছে কিনা তা পরীক্ষা করে দেখা হবে। এছাড়াও রক্ত ও মল পরীক্ষা করে আপনার শিশুর রোগ প্রতিরোধ ক্ষমতা নির্ণয় করা হবে।

আপনার সম্মতি থাকলে আমরা আপনার শিশুর জন্য নিম্নলিখিত ব্যবস্থা গ্রহণ করবো:

(১) আপনার শিশুর অসুখের ব্যাপারে জিজ্ঞাসাবাদ করা হবে এবং পূর্ণ শারিরিক পরীক্ষা করা হবে।

(২) একবার আপনার শিশুর পায়খানা সংগ্রহ করা হবে।

(৩) আপনার শিশুর হাতের শিরা থেকে ১মি:লি: (১চামচের ৫ভাগের ১ভাগ) রক্ত সংগ্রহ করা হবে। এই স্বল্প পরিমাণ রক্ত সংগ্রহ আপনার শিশুর জন্য সম্পূর্ণ নিরাপদ।

(৪) আমরা আপনার শিশুর শারিরিক ও সকল প্রকার পরীক্ষার ফলাফল গোপন রাখবো। কেবলমাত্র গবেষণায় সম্পৃক্ত চিকিৎসক, যুক্তরাষ্ট্রের ন্যাশনাল ইনস্টিটিউটের (যারা এই গবেষণা পরিচালনার জন্য আর্থিক সহায়তা প্রদান করছে) মনোনীত প্রতিনিধি এবং মনোনীত যথার্থ শাস্ত্রীয় প্রয়োগ নির্ণয়াকরারই (ইআর.সি-এথিক্যাল রিভিউ কমিটি) এই গবেষণার ফলাফল জানতে পারবে। সকল ফলাফল একটি লকারে রাখা হবে। আপনি চাইলে গবেষণার ফলাফল আপনাকেও জানানো হবে, তবে গবেষণার কিছু ফলাফল গবেষণা শেষ হওয়ার অনেক পরে পাওয়া যাবে।

(৫) একমাত্র আপনিই এই গবেষণায় আপনার শিশুর অংশগ্রহনের ব্যাপারে সিদ্ধান্ত নিতে পারেন। গবেষণা চলাকালীন যে কোন সময়ে আপনি সম্মতি প্রত্যাহার করতে পারবেন। এই গবেষণায় অংশগ্রহন না করলে, এমনকি অংশগ্রহনের পর সম্মতি প্রত্যাহার করলেও আপনার শিশু এই হাসপাতালের প্রচলিত সুচিকিৎসা থেকে বঞ্চিত হবে না।

(৬) এই গবেষণায় আপনার শিশুর অংশগ্রহনে আপনার সম্মতি থাকলে অনুগ্রহ করে নিচের নির্দিষ্ট স্থানে আপনার স্বাক্ষর অথবা টিপসই দিন

আপনার সহযোগিতার জন্যে ধন্যবাদ।

অভিভাবকের স্বাক্ষর অথবা টিপসই

তারিখ

গবেষকের স্বাক্ষর

তারিখ

সাক্ষীর স্বাক্ষর

তারিখ

গবেষকের নাম, পদবি ও যোগাযোগের টেলিফোন নম্বর:

ডা: ওয়াসিফ আলী খান

সহকারী বিজ্ঞানী

কলোরা হাসপাতাল

মহাখালি, ঢাকা-১২১২

ফোন-৯৮৮৬৭৩৪(সরাসরি)

৮৮১১৭৫১-৬০/২৩৩৩(সম্প্রসারণ)

ABSTRACT SUMMARY FOR THE ETHICAL REVIEW COMMITTEE

1. Requirement of study population

The aim of the study is to examine the incidence of various genotypes of *C. parvum* and correlate them with clinical findings (demography, duration of illness, severity of illness, etc) and immune responses in children ≤ 5 years of age coming to ICDDR, B with persistent diarrhoea.

2. Potential risk

The only risk to the patients is the rare possibility of infection consequent to venipuncture (venipuncture will be performed only once and only 1 ml of blood (1/5 tsp) will be collected. Venipuncture is a standard medical procedure, minor risks include momentary pain during insertion of the needle, a small risk of leaking of blood from the puncture site, and a rare chance of infection.

3. Methods for monitoring potential risks

Study patients will be interviewed, and a thorough physical examination will be performed. Vital signs of the patients will be recorded by the investigator. These procedures will ensure careful monitoring of the clinical condition of the patients and detection of potential adverse events.

4. Methods for safeguarding confidentiality

Patient information will be kept under lock, and the computer database on the study subjects will not include the name of the patients.

5. Informed consent

At the time of screening, study ward attendant(s) will inform the patients on the nature of the study, and their benefits and potential risks. The investigator will then obtain informed consent.

6. Interview

A brief, one page history form will be completed by the investigator. Completion of the questionnaire will require approximately 10 minutes. The questions do not depart from the questions that would be asked to make a clinical diagnosis under the usual circumstances.

7. Benefits to individuals and society

The benefits to the patient are that investigator who is well trained for conduction of such studies will examine them, and they would have thorough investigation done for their medical problems. The benefits to the general population are that important information may be derived to control the spread of *C. parvum* infection among children in developing countries and also management guidelines for the treatment of *C. parvum* infection.

8. Samples required

The study will require use of blood and stool samples.



INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH
Mail : ICDDR,B. GPO Box 128, Dhaka-1000, Bangladesh
Phone : 871751-60, Telex : 675612 ICDD BJ
Fax : 880-2-883116, 886050, 871568, 871686. Cable : Cholera Dhaka

Memorandum

To: Chairman
Research Review Committee
Date: August 6th 2000

Through: Associate Director
Clinical Sciences Division

From: Dr. Wasif Ali Khan
Principal Investigator

Subject: Research Protocol entitled: " Molecular Epidemiology of Cryptosporidiosis".

I am pleased to enclose herewith our response to the comments raised by the external reviewers of the abovementioned proposal, for kind consideration by the Research Review Committee.

Reviewer A

Didn't have any specific comments and supports to conduct the study.

Reviewer B

We appreciate Dr. Butterton's thoughts. The specificity of a modified acid fast stain for detecting *Cryptosporidium parvum* in a single stool approaches 100% in experienced hands (as is the case with the microbiologists at the ICDDR, B). The sensitivity is approximately 70% in comparison to immunofluorescent antibody detection techniques (Alles AJ et al, J Clin Micro 1995;33:1632-34) and PCR techniques. Patients identified as having cryptosporidiosis by routine microscopy will, therefore, indeed have the infection. Samples from control patients will be analyzed in Bangladesh by routine microscopy and then will be confirmed as being negative by both immunofluorescent staining and PCR techniques in Boston. With an incidence of cryptosporidiosis of approximately 2% at the ICDDR, B and a sensitivity of routine microscopy of 70%, it would be predicted that only 1-2 of the 50 control patients would subsequently be identified as having cryptosporidiosis when analyzed in more detail in Boston. We, therefore, do not expect a significant dropout rate of control samples.

As mentioned by Dr. Butterton, preliminary studies have shown that some stool samples with *C. parvum* test PCR negative (probably because of

inhibitors in stool specimens). Our proposed study is a pilot project that will allow us to better quantitate this rate. Even with a possible negative PCR rate of approximately 10% (as previously found), we would expect to be able to evaluate the genotypes of over 130 isolates from those continents in our pilot project as currently proposed.

We sincerely hope that the Committee would consider approval of the protocol.

Thank you.

REVIEWER-A

Page 1 (of 2)

Title: Molccular epidemiology of cryptosporodiosis

Summary of the Referee's Opinion: Please see the following table to evaluate the various aspects of the proposal by checking the appropriate boxes. Your detailed comments are sought on a separate, attached page.

	Rank Score		
	High	Medium	Low
Quality of project	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Adequacy of project design	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Suitability of methodology	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Feasibility within time period	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Appropriateness of budget	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Potential value of field of knowledge	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

CONCLUSIONS

I support the application:

- a) without qualification / /
- b) with qualification
 - on technical grounds / /
 - on level of financial support / /

I do not support the application / /

Name of the Referee: Elizabeth Hohmann, M.D.

Signature: *Elizabeth Hohmann*

Date: 8/4/00

REVIEWER-A

Position: Assistant Professor, Harvard Medical School.....

Institution: Division of Infectious Diseases, Massachusetts General Hospital, Boston MA

Detailed comments

Please briefly provide your opinions of this proposal, giving special attention to the originality and feasibility of the project, its potential for providing new knowledge and the justification of financial support sought; include suggestions for modifications (scientific or financial) where you feel they are justified)

(Use additional pages if necessary)

Title: Molecular epidemiology and humoral immune responses to *Cryptosporidium parvum*.

PI: Wasif Ali Khan

Reviewer:

This is a useful study with standard techniques which will add to knowledge about this chronic but rather poorly studied diarrheal illness. The inclusion of several different geographic sites is a strong feature. The investigators are experienced and knowledgeable. The budget is modest. I have no reservations about endorsing this project.

REVIEWER-B

Page 1 (of 2)

Title: Molecular epidemiology of cryptosporidiosis

Summary of the Referee's Opinion: Please see the following table to evaluate the various aspects of the proposal by checking the appropriate boxes. Your detailed comments are sought on a separate, attached page.

	Rank Score		
	High	Medium	Low
Quality of project	✓		
Adequacy of project design	✓		
Suitability of methodology	✓		
Feasibility within time period	✓		
Appropriateness of budget	✓		
Potential value of field of knowledge	✓		

CONCLUSIONS

I support the application:

- a) without qualification ✓
- b) with qualification
 - on technical grounds / /
 - on level of financial support / /

I do not support the application / /

Name of the Referee: Joan R. Buterton, M.D.

Signature: Joan Buterton

Date: 8/4/00

REVIEWER-B

Position: Assistant Professor, Harvard Medical School.....

Institution: Division of Infectious Diseases, Massachusetts General Hospital, Boston MA

Detailed comments

Please briefly provide your opinions of this proposal, giving special attention to the originality and feasibility of the project, its potential for providing new knowledge and the justification of financial support sought; include suggestions for modifications (scientific or financial) where you feel they are justified)

(Use additional pages if necessary)

Title: Molecular epidemiology and humoral immune responses to *Cryptosporidium parvum*.

PI: Wasif Ali Khan

Reviewer:

Molecular epidemiology of cryptosporidiosis

Summary: This application proposes a one-year pilot study to investigate the molecular epidemiology and host antibody responses to *Cryptosporidium parvum* infection in children in Bangladesh. *C. parvum* is a major pathogen in children with acute and persistent diarrhea, yet little is known about possible correlations between organism genotype and clinical or epidemiological features of disease. The investigators are requesting a very modest budget for clearly defined objectives, and the proposed studies have great potential for providing new information about this important pathogen. The unique opportunity afforded by the interaction between the ICIDR investigators in this pathogen specific group will allow these experiments to have a broad scientific impact. The ICDDR, B site is ideal for the proposed experiments, having the necessary patient base and laboratory facilities needed to conduct the study. The impact on patients is minimal, and has the potential benefit of providing a detailed stool examination to the patients that are seen.

Specific comments:

1. The specificity and sensitivity of a single modified acid fast stain examination of stool in detecting *C. parvum* should be provided, particularly given that this study forms the basis of selecting cases and controls.
2. What is the likelihood that usable DNA will be isolated from stool samples found to be positive for *C. parvum* by microscopy? In the South Africa study discussed, PCR failed in 3 out of 24 (12.5%) of HIV-infected children with diarrhea for more than 7 days who had positive stool samples. It is possible that there is a higher *C. parvum* burden in these patients than in non-HIV-infected children with acute diarrhea. Was the sample size of 50 determined with at least this rate of PCR failure taken into account?



INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH

Mail : ICDDR,B, GPO Box 128, Dhaka-1000, Bangladesh

Phone : 871751-60, Telex : 675612 ICDD BJ

Fax : 880-2-883116, 886050, 871568, 871686, Cable : Cholera Dhaka

Date : August 29, 2000

To : Chairman ERC

From : Dr Nigar S. Shahid *Nigar S Shahid*

Subject : **Review of protocol # 2000-22 entitled "Molecular Epidemiology of Cryptosporidiosis"**

Thank you for sending the above protocol for review.

✓ Answer to Q5b should read No as the subjects will be minor and the study will require consent from guardian.

It is not clear who the PI is (Fuchs or Khan) (Page 3 of cover page).

The study entails identifying children (< 5 years of age) with *Cryptosporidium* examine its genotypes, correlate this information with geographic location, age, clinical severity of illness, drinking water source and contact with animals. It also entails estimation of serum and faecal antibodies to the organism.

The protocol is straight forward and there are no ethical issues involved and as such it could be recommended.