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Item No. 12.

Date 14.7.83

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Dr.S.A. Selim

Application No. 83-031(P)

Title of Study 'A study of the prevalence of rotavirus infection in calves in the selected areas of Bangladesh'

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 form)

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

1. Source of Population:
   (a) Ill subjects ** (Yes) No
   (b) Non-ill subjects (Yes) No
   (c) Minors or persons under guardianship Yes (No)
2. Does the study involve:
   (a) Physical risks to the subjects Yes (No)
   (b) Social Risks Yes (No)
   (c) Psychological risks to subjects Yes (No)
   (d) Discomfort to subjects Yes (No)
   (e) Invasion of privacy Yes (No)
   (f) Disclosure of information damaging to subject or others Yes (No)
3. Does the study involve:
   (a) Use of records, (hospital, medical, death, birth or other) (Yes) No
   (b) Use of fetal tissue or abortus Yes (No)
   (c) Use of organs or body fluids Yes (No)
4. Are subjects clearly informed about:
   (a) Nature and purposes of study Yes No
   (b) Procedures to be followed including alternatives used Yes No
   (c) Physical risks Yes No
   (d) Sensitive questions Yes No
   (e) Benefits to be derived Yes No
   (f) Right to refuse to participate or to withdraw from study Yes No
   (g) Confidential handling of data Yes No
   (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure Yes No

   (NA)
5. Will signed consent form be required:
   (a) From subjects Yes No (NA)
   (b) From parent or guardian (if subjects are minors) Yes No (NA)
6. Will precautions be taken to protect anonymity of subjects Yes No (NA)
7. Check documents being submitted herewith to Committee:
   ___ Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
   ✓ Protocol (Required)
   ✓ Abstract Summary (Required)
   ___ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
   ___ Informed consent form for subjects
   ___ Informed consent form for parent or guardian
   ___ Procedure for maintaining confidentiality
   ___ Questionnaire or interview schedule *

* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:
1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will be presented to the Cttee. for review.

** The study involves the animal (calf) population only.

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

S. A. Selim
Principal Investigator

Trainee

83-031(P)
24.7.83

SECTION- I

RESEARCH PROTOCOL (PILOT)

1. Title: A study of the prevalence of rotavirus infection in calves in the selected areas of Bangladesh.

2. Principal Investigator: Dr. Sheikh Abdus Selim, D.V.M.

   Supervisors: Dr. A. Jalil Sarker (BAU) &
   Dr. K.M.S. Aziz (ICDDR-B).

   Consultant: Dr. A.R. Samadi.

3. Starting Date: August, 1983

4. Completion Date: January 1984 for the pilot protocol.

5. Total Direct Cost: US $ 2060

6. Scientific Programme Head: Dr. A.R. Samadi.

This protocol has been approved by the Disease Transmission Working Group.

A Samadi

Signature of the Programme Head

Date: 18/7/1983

7. Abstract Summary

Rotaviruses are a common and worldwide cause of neonatal gastroenteritis in both humans and animals. Bovine rotavirus has a prime importance in calf morbidity and mortality, in association with diarrhoea. The virus is morphologically similar and antigenically closely related to the human rotavirus.

Fecal specimens will be collected from diarrhoic and also from non-diarrhoic calves in selected dairy farms and villages where they are reared conventionally, all belonging to the age group of birth to 1 year. The samples will be examined for the detection of calf rotavirus by ELISA technique. Only some of the positive samples (approx. 10%) will be re-examined by direct electron microscopy for identification and demonstration of the virus and for comparison and confirmation of the results.

8. Reviews:

a) Research Involving Human Subjects: _____

b) Research Committee: _____

c) Director: _____

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SECTION- II

RESEARCH PLAN

(A) Introduction.

1. Objective: Acute infectious gastroenteritis is generally a common disease in young mammals. Rotaviruses have been considered as one of the important common causes of such disease that are worldwide in distribution in the youngs of both humans and animals. In Bangladesh, human beings and animals have a close association. Human and bovine rotaviruses are antigenically closely related. Under natural conditions, it is possible that human contacts could be a source of rotavirus infection for animals and vice versa.

In the present study we aim to determine the extent of existence of rotavirus in the young calves in the selected areas of Bangladesh. Once ratavirus is detected initially through ELISA test, electron microscopy will be carried out for direct visualization of the virus.

2. Background: The earliest research on rotaviral infection in animals (epidemic diarrhoea in new born) were carried out by Light and Hodes[1] with the transmission of diarrhoea to calves with filtrates of feces from human infant that had diarrhoea. In 1969, Mebus et. al. demonstrated virus like particles/filterable agents in the diarrhoic feces of calves[2]. Medical virologists found simillar particles in the feces of infants and young children in 1973[3-6]. In 1976, the filterable agent concerned was shown to be rotavirus.[7]

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At present, the evidences available reveal that rotaviruses are pathogenic to a wide variety of young mammals including children and calves and are probably the most important infectious agent associated with enteritis[8-10]. Rotavirus infection was initially recognized as a veterinary problem[2,11] and subsequently as a child health problem[3-5]. From virological and serological studies it is evident that infections occur in early life in 90-100% calves and pigs as is the case in man, and most strains irrespective of their host, at least under experimental conditions, show cross infection between mammalian species [12].

Diarrhoeal diseases due to viruses in animals are reported since long[1,2,13-15] and the role of rotaviruses in diarrhoea, particularly in the young calves has been established[8,9,16-22]. The incidence of diarrhoeal diseases of the young calves seems to be considerably high as indicated from the records of dairy farms and veterinary hospitals of Bangladesh[23]. Recently, the presence of rotaviruses in calf feces has been reported in a few diarrhoic calves of Bangladesh[24]. As calf diarrhoea ("calf scour") in Bangladesh might be a serious challenge to any livestock owner and no scientific work has yet been conducted with a specific aim regarding the involvement of viral agent to such disease in calves, as such a preliminary work in this respect is undertaken. The present research plan mostly based on the suggestions given by two leading scientists in this field[25,26] and also in collaboration with the Supervisors. (Drs. K.M.S. Aziz and A. Jalil Sarker).

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3. RATIONALE

Rotavirus infection in calves and in children as an etiologic agent of diarrhoea is one of the commonest veterinary as well as child health problem throughout the world. The association between man and animals in Bangladesh is very close where they share the same premises. The rotavirus infection in human in Bangladesh, as it has been doubted,might be of bovine origin (Dr. C.A. Mebus, personal communication). This study will cover the extent of rotavirus infection in calf population in this part of the world as well as will open the field of research in this aspect which will be of great helpful in formulation of its probable zoonotic importance and of preventive measures at the national level.

(B) SPECIFIC AIM.

To study the prevalence of rotavirus infection in calves up to one year of age in selected farms and villages of Bangladesh.

(C) METHODS OF PROCEDURE

The areas selected for experimental purpose are situated in two different regions of Bangladesh, viz. Dhaka and Mymensingh districts. The spots for sample collection are: (1) The Dairy and Cattle Improvement Farm, Savar, Dhaka; (2) Millitary Dairy Farm, Savar, Dhaka; (3) B.A.U. Dairy Farm, Mymensingh; (4) Two villages adjacent to the BAU Campus - Baira and Kewatkhali, Mymensingh.

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Approximately 1000 fecal specimens will be collected from calves with and without diarrhoea of age group from birth to 1 year. 50% (i.e. 500) of the total samples will be taken from diarrhoic calves that is calves showing frequent discharge of liquid feces and will be compared with 500 calves without diarrhoea as controls. The diarrhoeal cases in each farm or village will be matched with control by age respectively. One sample from each individual at its diarrhoeal stage will be the rule. Each experimental area will be visited once a week for collecting of samples.

Hand equipped with sterile glove will be used for collecting the samples through inserting the fingers into the rectum of calves. After collection from the rectum with all precautionary measures, the fecal material will be kept in sterile, screw topped, secure container that contains PBS. The collected samples will be transported to the lab. and will be centrifused at 3000 r.p.m. for 20 minutes and the supernatent will be kept in deep freezer ($-20^{\circ}C$) until ELISA is performed with further processing.

ELISA test for calf rotavirus will be performed in the ICDDR,B Lab. with standard ELISA technique.[27,28] In the antibody coated microplate wells the processed fecal extracts, enzyme labelled specific antibody and enzyme substrate will be added successively, each with a definite interval and washing step(s). Final result will be measured in a titer tek.

Electron Microscopy (EM) with a few positive samples, will be carried out by adopting standard technique[29] or the technique followed by the Armed Forces Institute of Pathology and Transfusion (AFIPT), Dhaka for the same. Interpretation of the result will be drawn after keen observation of the viral morphology.

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(D) SIGNIFICANCE

This study will lead to a better understanding of the viral agent in animal (calf) population of Bangladesh as a cause of diarrhoeal diseases which will help us to study its probable transmission to human beings.

(E) FACILITIES REQUIRED

1) Laboratory space - available in the training laboratory of ICDDR,B.

2) Logistic support - transport for sample collection.

3) ELISA test - the Principal Investigator (PI) himself will perform the ELISA test in the ICDDR,B Laboratory with the supplied material, through this protocol. For this the PI will undergo training for ELISA test and will take part in the ELISA tests of the ongoing protocols under the supervision of Mr. S. Huda, Immunology Branch, ICDDR,B.

4) Electron Microscope - available at the AFIPT, technical support for this will be kindly provided by the AFIPT.

REFERENCES

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SECTION - III

A. DETAILED BUDGET

1. PERSONNEL

Name	Position	%	Annual salary	Project requirement
Dr. Sheikh Abdus Selim	Principal Investigaror	75	-	-
Dr. A. Jalil Sarker	Associate Professor	10	-	-
Dr. K. M. S. Aziz	Associate Director	5	-	-
Dr. A. R. Samadi	Scientific Program Head	-	-	-

2. SUPPLIES & MATERIALS

Items	Amount	Unit cost	Project requirements
1) Sample collecting bottles	144x10	$ 20.74/144	US$ 210
2) Disposable hand gloves & insulating foam box	(1200+1)	$ 3.67/100 & 28.44	US$ 75
3) ELISA test (with re-agent & materials)	1000	$ 1.00	US$ 1000

3. EQUIPMENTS — As above

4. PATIENT HOSPITALIZATION — None

5. OUTPATIENT CARE — None

6. ICDDR,B TRANSPORT

A weekly return visit - (ICDDR,B to Savar) Tk. 4.50 (50x26) US$ 250
(26 weeks, 50 miles)

7. TRAVEL & TRANSPORT OF PERSONS

8. TRANSPORTATION OF THINGS

1 (one) person from Dhaka to Mymensingh with necessary things and equipment by Train and from there to the experimental areas by road Tk. 2(85+45)x 26 return trips. US$ 275

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9. RENT, COMMUNICATION & UTILITIES		None
10. INFORMATION SERVICES		US$ 50
11. PRINTING & REPRODUCTION		100
12. OTHER CONTRACTUAL SERVICES		100
	Total	US$ 2060

B. BUDGET SUMMARY

	Category	US $
1.	Personnel	None
2.	Supplies & Materials	1285
3.	ICDDR,B Transport	250
4.	Travel & Transportation of persons and things	275
5.	Informations services	50
6.	Printing & Reproduction	100
7.	Other contractual services	100
	Total	US $2060

APPENDIX - I

Table - 1

SAMPLE SELECTION*

Age group	Diarrhoic	Non-Diarrhoic	Total	Sources	
				Farms	Villages
Birth - 5 weeks	200	200	400	300	100
5 weeks - 3 months	100	100	200	150	50
3 months - 6 months	100	100	200	150	50
6 months - 1 year	100	100	200	150	50
Birth - 1 year	500	500	1000	750	250

Table - 2

ELISA FOR ROTAVIRUS IN FARM CALVES

Age	Diarrhoic			Control (Non-Diarrhoic)		
	Total	Rota		Total	Rota	
		+	-		+	-
Birth - 5 w						
5 w - 3 m						
3 m - 6 m						
6 m - 1 year						
Birth - 1 year						

*Variable with the occurrence of the diarrhoeal cases

APPENDIX -II

Table - 3

ELISA FOR ROTAVIRUS IN COMMUNITY (VILLAGE) CALVES

Age	Diarrhoic			Control (Non-Diarrhoic)		
	Total	Rota		Total	Rota	
		+	-		+	-
Birth-5 weeks						
5w - 3 months						
3m - 6 m						
6m - 1 year						
Birth - 1 year						

Table - 4

ELISA VS. EM FOR POSITIVE CASES

	ELISA	EM	
	All positive	+	-
Diarrhoic			
Non-Diarrhoic			
Total			