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Attachment 1.

Date 16.11.83

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Dr Habibur Rahman

Trainee Investigator (if any)

Application No. 83-040(A)

Supporting Agency (if Non-ICDDR,B)

Title of Study Diarrhoea due to enterotoxigenic and enteropathogenic E. coli in calves in Bangladesh.

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

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

Habibur Rahman
Principal Investigator

Trainee

RESEARCH PROTOCOL (PILOT)

SECTION - I

(1) Title: Diarrhoea due to enterotoxigenic and enteropathogenic E. coli in calves in Bangladesh.

(2) Principal Investigator: Dr Habibur Rahman

Supervisors: Dr M I. Huq
Dr M. Mansurul Amin

(3) Starting Date: Ist September, 1983

(4) Completion Date: 28th February, 1984

(5) Total Direct Cost: US$ 1,972.91

(6) Scientific Program Head

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

Signature of the Scientific Program Head K. M. S. Aziz

Date The 2nd November 1983

Abstract Summary

In Bangladesh the incidence of diarrhoeal diseases in animals are high and a large number of animal population die of this disease annually. A major percentage of the causative agents are bacteria followed by virus and parasite. There are about 46 main aetiologic agents that are responsible for causing diarrhoea in domestic animals. The protocol is designed to look at the incidence of diarrhoeal disease in calves due to enterotoxigenic and enteropathogenic E. coli in two districts of Bangladesh i.e. Dhaka and Mymensingh and to characterise them. The study period will from September, 1983 to 28th February, 1984.

SECTION - II

RESEARCH PLAN

A) INTRODUCTION

There is a high morbidity & mortality due to diarrhoea amongst calves in Bangladesh. Very little work has been done on aetiology of this disease in this country. As enterotoxigenic and enteropathogenic Escherichia coli is one of the major causes of diarrhoea in calves in other countries - E. coli and Salmonellae were recognised as the most common bacterial causes of neonatal calf diarrhoea in Britain (E.A. Fisher & A.A. Martinez(1976) (1) R.R. Al-Mashat & D.J. Taylor - (1983) (2) .

It is expected that the same may be there in Bangladesh, we therefore intend to look for the incidence of E. coli diarrhoea in calves in this country.

B) BACKGROUND

Janson (1895) was the first to point the importance of E. coli as a potential pathogen in calves. Since then its association with different disease conditions were studied by Khera & Dhanda (1963) (5) and Verma & Adlakha (1970). (4)

E. coli causes diarrhoea both in animals & human beings either by invasion of intestinal mucosa or by the elaboration of enterotoxins - Dupont et al (6) 1971, Isacson et al 1978 (7), Moon 1974 (8), Smith et al - 1967. (9).

The enterotoxigenic strains of E. coli (ETEC) is reported to cause diarrhoeal disease in several species of animals (Moon et al 1974) (Smith et al 1967).

Smith et al (1967) (9) observed that enteropathogenic E. coli strains were able to dilate isolated segments of calf intestines.

It was recognised by Huq et al(1979)[13] that certain strains of Escherichia coli produce an enterotoxin similar to cholera enterotoxin which causes fluid accumulation in ligated ileal segment of adult rabbit's & some other animals. It was further determined that the enterotoxigenic strains of E. coli elaborate one or both of two plasmid mediated enterotoxins.

(i) High molecular weight heat labile enterotoxins (LT) similar to cholera toxin which acts by the stimulation of adenylate cyclad

and (ii) A low molecular weight heat stable enterotoxin (ST) which acts by the stimulation of Guanylate Cyclase (4,5).

The most common diseases experienced upto 12 weeks in diseased calves were studied by Andrews & Read (1983)[16] in which:

1. respiratory diseases - 31.2%
2. diarrhoeas - 20.1%
3. eye infections - 11.20%
4. navel & joint problems - 2.2%

In total 269 calves were taken for study & 3 died.

The haemolytic E. coli were the usual isolates from rectal swabs. No Salmonella spp. were present.

Pathogenesis of ETEC mediated diarrhoea has been shown to be dependent upon (1) the ability of the organism to colonize & proliferate in the part of the small intestine facilitating the attachment to the mucosa of small intestine by its pile (2) the ability to elaborate enterotoxin capable of inducing copious intestinal secretion

Further, the enterotoxins produced by some strains enteropathogenic for pigs and calves and human being have been demonstrated to be plasmid controlled. The particular plasmid involved being designated as Ent. plasmids are non-chromosomal genetic elements of certain bacteria which can be transferred from one bacterial strain to another by sexual conjugation. Smith (1967) [9]

Gay (1965) [17] delineated three distinctive syndromes in new born claves which he attributed to Escherichia coli

(1) The first syndrome, he characterised in his experiment coli- septicaemis, occurs in the first week of life causing invasion of the it tissues of the intestine, menifests by fever & collapse & cause death.

(2) The second form so called enterotoxic coli bacillosis, occurs in the first week of life & is characterised by acute diarrhoea but no systemic invasions which may cause death.

The third form, enteric coli bacillosis, occurs mainly after first week of life & may be described as the traditional "which score" of calves.

Fisher et al (1975) [10] conducted an experiment on fluid balance of calves with a defined enteric coli bacillosis and demonstrated that when fluid input was maintained there was no significant difference between the total fluid output of healthy nondiarrhoeaic calves, surviving diarrhoeaic calves and dying calves.

Gyles et al (1978) [18] conducted an experiment to transfer of an enterotoxin (Ent.) Plasmid from a procine entero pathogenic E. coli to E. coli K12 strain in the intestine of newly weaned pigs. The Ent. plasmid carried genes for resistance to tetracycline, streptomycin & sulfonamides.

The enteropathogenic gut dilating serotypes of E. coli had been isolated in Great Britain and United States by Smith & Halb's 1967a), In Canada by Gay, McKay & Barnum 1964 in Switzerland by Corboz & Becker 1973 and in Belgium by Schoenaers & Kaeckeen beck 1973. E.W. Fisher & A.A. Martinez - (1975).(10)

The ETEC strains are known to produce two types of enterotoxin (Smith et al 1970).(11) One type is characterised as a larger molecular weight - heat labile, immunogenic toxin and secretory response on intestinal mucosa is delayed in onset and of long duration.

The second type is of smaller molecular weight, heat stable toxin that is apparently non immunogenic and the response is rapid in onset and of short duration.

E. coli & Salmonellae were recognised as the most common bacterial causes of neonatal calf diarrhoea in Britain. Vety. Invest. Service, 1964 - (E.W. Fisher (1976))(12)

Pearson et al (1978)(19) infected three neo-natal calves and E. coli type = O101K(A) protected from coli septicaemis by intravenously administered immunoglobulin M. Severe enteric coli bacillosis was developed in all the three calves. In the distal half of small intestine stunting and fusion of villi were seen at four days.

METHOD & PROCEDURES:

1. The study will be carried out in the diary farms at Savar, Dhaka and Agricultural University, Mymensingh.

2. Subjects - Calves within one year of age.

3. Number of specimens - 500, divided into two groups. Each group will have 150 diarrhaic calves and 100 normal calves.

4. Collection of samples: The faecal samples of diarrhoea calves will be collected by hand with globes from the rectum. Aseptically the sample will be collected in sterile universal container and transported to the laboratory in ice in a foam box and processed for bacteriology within 6 hours at ICDDR,B Laboratory.

   Or samples will be plated at the site of collection and brought to the laboratory for incubation.

5. Culture: The samples will be plated onto MacConkey Agar along with other diagnostic media such as SS Agar, TTGA media & Brucella agar and incubated overnight at 37°C Bile peptone medium and selenite F broth are used for enrichment of V. cholerae, Salmonella & Shigella. Enrichment broths are plated onto MacConkey, SS Agar and TTGA. Biochemically confirmed two typical E. coli colonies are stocked in a Blood Agar base slant in 1 drum screw cap culture vial and kept at room temperature (M.I. Huq et al, 1979)[13].

6. The enteropathogenicity & enterotoxigenicity test will be done from these slants:

   a. Enteropathogenicity: Slide agglutination test will be done with commercially available antisera. The antisera available in ICDDR,B laboratory are of following Ø serotypes:

1. E. coli (OK) antisera Poly A
   026: K60, 055; K59
   0111:K58, 0127a:K63

2. E. coli (OK) antisera Poly B
   08ba;K61, 0119:K69,0124:k72
   0125:K70,0126:K71,0128:K67

3. E. coli (OK) antisera Poly C
   018a018c:K77, 020a20c:K61, 020a20b:K84
   028:K73,044:K74,0112a0112c:K66.

b. Enterotoxigenicity: E. coli strains are inoculated into 2.5 ml of Trypticase Soy Broth + 0.6% Yeast Extract medium already sterilized in tubes suitable for fit in a roller drum. For each run control toxin positive and a control toxin negative strains are inoculated. Cultures are incubated at 37°C in a roller drum running at a speed of about 18-20 circles per minute.

After about 20-22 hrs. incubation the tubes are taken out and centrifuged in a sorval refrigerated centrifuge for 15-20 min at 13000 rpm using SM-24 type head & plastic tubes. The supernate liquid is devided equally in two vials. In one vial a drop of gentamycin solution is added which will be used for ST assay. The other tube is kept frozen at 60°C and is used for Chinese hamster ovary cell assay (M.I. Huq et al, 1979)[13].

SIGNIFICANCE:

This study will lead to a better understanding of the diarrhoea aetiology of calves in Bangladesh especially the role of E. coli. This will be of help in formulating preventive measures for calf diarrhoea.

RATIONALE

Very little is known about the problem in Bangladesh. E. coli is one of the commonest aetiologic agent of diarrhoea in calves all over the world. This study may elucidate whether that is true for this country as well and if so what is the role of ETEC & EPEC strains. Then informations are important to formulate national plans for reduction of morbidity and mortality in calves.

SPECIFIC AIM:

To study the prevalence of diarrhoea in calves due to enterotoxigenic and enteropathogenic E. coli upto 1(one) year of age.

E. FACILITIES REQUIRED

1. Laboratory space
2. Logistic support
3. Research work

REFERENCES

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3. Jenson C.O. (1893). Mh. Tlerlleik 4:97. Cited by Srivastava, N.C. (1979) - Indian Vet. J. 56: 901-903.

4. Verma K.C. & Adlakha S.C. (1970). E. coli infections in calves. Indian J. Anim. Health - 9: 107.

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7. Isaacson, R.E., Moon H.W. & Schneider R.A. (1978). Distribution and viraland of E. coli in the small intestine of calves with & without diarrhoea. Am. Vety. Res. 1750-1755.

8. Moon H.W. (1974) - Pathogenesis of enteric diseases by E.coli Adv. Vety. Sci. Comp. Med. 18: 179-211.

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14. Ataur, R. (1972): Incidence of diseases of cattle in Mymensingh. Bangladesh Vety. J. 6: 25-30.

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18. Gyles, C. Falkow, S.R. (1978). In vivo Transfer of an E. coli Ent. toxin plasmid possessing genes for drug resistance. Am. J. Vety. Res. 1438-1441.

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SECTION III

BUDGET

A. PERSONNEL

Name	Position	% of work	Annual salary	Project requirement Taka	US $
1. Dr Habibur Rahman	Principal Investigator				
2. Dr M. Monsurul Amin,	Supervisor				
3. Dr M.I. Huq	Supervisor				1,300.00

B. SUPPLEMENTS AND MATERIALS

1. Media Tk.9,000.00

2. Glassware & petridishes Tk.8,000.00

3. ST & LT assay 600x4 Tk.8,400.00

C. EQUIPMENTS: None

D. PATIENT HOSPITALIZATION: None

E. OUTPATIENT CARE: None

F. TRANSPORT

Land transport - ICDDR,B to Savar
(a weekly return visit)
700 miles Tk.3,150.00

G. TRAVEL AND TRANSPORT OF PERSONS

From ICDDR,B to Mymensingh Tk.4,800.00
x 12 visits with other expenses

H. TRANSPORTATION OF THINGS: None

I. RENT COMMUNICATION None

J. INFORMATION SERVICE None

K. PRINTING AND REPRODUCTION Tk.2,000.00

L. OTHER CONTRACTUAL SERVICE Tk.12,000.00

B. BUDGET SUMMARY

		TAKA	US DOLLAR
1.	PERSONNEL SERVICES	-	1 300.00
2.	SUPPLIES MATERIALS	25,400.00	
3.	EQUIPMENT	None	
4.	PATIENT HOSPITALIZATION	None	
5.	OUTPATIENT CARE	None	
6.	LAND TRANSPORT ICDDR,B to Savar 700 miles	3,150.00	
7.	TRAVEL AND TRANSPORT OF PERSONS From ICDDR,B to Mymensinghx12 visits and other expenses	4,300.00	
8.	TRANSPORTATION OF THINGS	None	
9.	RENT COMMUNICATION	None	
10.	INFORMATION SERVICE	None	
11.	PRINTING AND REPRODUCTION	2,000.00	
12.	OTHER CONTRACTUAL SERVICE	12,000.00	
	Sub-Total =	47,350.00	1,300.00

Dollar 1,972.91 Dollar 1,300.00

Total Direct Cost US$ 1,972.91

1 dollar = Tk.24.00

SAMPLE COLLECTION SHEET

Serial No.

1. Collection Date: _____ Time _____

2. Farm/Village _____ Location _____

3. Tag No./Brief description of the calf _____

_____

(a)

Age	Date of Birth
Y	Y
M	M
D	D

(b)

Sex	♂	♀

(c)

Breed			
Ind	Cross	Jursy	SW

(d)

Suckling/Harbivorous

4. Diarrhoeal informations:

   (a) Recumbent/weak

   (b) Faeces - liquid/Mucoid/bloody

   (c) Colour of faeces

   (d) Onset: Date _____ Time _____

   (e) Duration - Days _____ hours _____

   (f) Treatment Drug _____ Course: _____

5. Past history of diarrhoea - yes/no

6. Faecal Exam. (if any): Date _____

   Report _____

7. Other informations: _____

   _____

_____

Signature of Investigator

Give "V" mark in appropriate word.

Protocol No.
Name of research: Diarrhoea due to entertoxigenic and enterpathogenic E.coli in calves in Bangladesh

Supervisor: Dr. M.I.Huq
Principal Investigator: Dr. Habibur Rahman

Sl. No.	Tag No.	Source	Microbio.No	E.coli		Campylo	Yarsina	Shigella	Salmonella	Vibrio
				ST	LT					

Lab No.

Bacteriological Rerport:

Date:

Requesting		
Div.	Br.	Proj.

No. of samples

Rate

Working		
Div.	Br.	Proj

P-4

INFANT MICE AND CHO CELL ASSAY FORM FOR E.COLI ST AND LT TOXIN

Name of the Investigator: Dr Habibur Rahman Project Code#: _____

Date of inoculation: _____

Sample #	Pool or Pick No.	Mice No.	Gut wt.	Remarks Body wt.	Ind Ratio	Av. Ratio	RESULTS ST	RESULTS LT	Comments
1		1							
		2							
2		1							
		2							
3		1							
		2							
4		1							
		2							
5		1							
		2							
6		1							
		2							
7		1							
		2							
8		1							
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9		1							
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10		1							
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11		1							
		2							
12		1							
		2							
13		1							
		2							
14		1							
		2							
15		1							
		2							
16		1							
		2							
17		1							
		2							
18		1							
		2							
19		1							
		2							
20		1							
		2							

SEROLOGICAL IDENTIFICATION OF EPEC

Protocol No.

Name of Investigator: Dr Habibur Rahman

Date:

Tag No	Source	Microbiological No	Polyvalent OK SERA	Monovalent OK SERA