

## ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Dr. Md. Imdadul Haq Trainee Investigator (if any) \_\_\_\_\_  
 Application No. SI-012 Supporting Agency (if Non-ICDDR,B) \_\_\_\_\_  
 Title of Study A study on the incidence of diarrhoeal disease in domestic animals of Bangladesh and its relationship to that in human being. 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).

- Source of Population:
- (a) Ill subjects Yes  No
- (b) Non-ill subjects Yes  No
- (c) Minors or persons under guardianship Yes  No
- Does the study involve:
- (a) Physical risks to the subjects Yes  No
- (b) Social Risks Yes  No
- (c) Psychological risks to subjects Yes  No
- (d) Discomfort to subjects Yes  No
- (e) Invasion of privacy Yes  No
- (f) Disclosure of information damaging to subject or others Yes  No
- Does the study involve:
- (a) Use of records, (hospital, medical, death, birth or other) Yes  No
- (b) Use of fetal tissue or abortus Yes  No
- (c) Use of organs or body fluids Yes  No
- Are subjects clearly informed about:
- (a) Nature and purposes of study Yes No
- (b) Procedures to be followed including alternatives used Yes No
- (c) Physical risks Yes No
- (d) Sensitive questions Yes No
- (e) Benefits to be derived Yes No
- (f) Right to refuse to participate or to withdraw from study Yes No
- (g) Confidential handling of data Yes No
- (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure Yes No
5. Will signed consent form be required:  
 (a) From subjects Yes  No   
 (b) From parent or guardian (if subjects are minors) Yes 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.
- The study involves only domestic animals. The laboratory stock cultures from patients will be compared.**

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

Imdadul Haq  
Principal Investigator

Trainee

SECTION I - RESEARCH PROTOCOL

- (1) Title: A study on the incidence of diarrhoeal disease in domestic animals of Bangladesh and its relationship to that in human being.
- (2) Principal Investigator: Dr. Md. Imdadul Huq, Head, Microbiology Branch, ICDDR,B
- Co-Investigator: Dr. Nitish Chandra Devnath  
DVM, Agricultural University, Mymensingh
- (3) Starting Date: March 15, 1981
- (4) Completion Date: March 14, 1982
- (5) Total Direct Cost: US \$ 15,909.00
- (6) Scientific Program Head:

This protocol has been approved by the DTWG

Working Group.

Signature of Scientific Program Head: Flanady

Date: 4/3/1981

(7) Abstract Summary:

In Bangladesh the incidence of diarrhoeal disease in animals are high and high percentage are of bacterial origin followed by diseases of parasitic origin. There are about 46 different diseases or manifestations which are responsible for causing diarrhoea in domestic animals. This study will cover the isolation and characterisation of the etiologic agents responsible for causing diarrhoea in domestic animals mostly in Dacca and Mymensingh. Samples will be collected from cattle, poultry, house hold pets etc. and will be looked for parasites followed by culturing for pathogens. The bacterial pathogens to be looked at are Vibrios, Salmonella, Shigella, Enterotoxigenic and Enteropathogenic E. coli, Aeromonas, Yersinia and Campylobacter. Among the Virus, only rotavirus will be looked at here and samples will be collected for Adinovirus. The antibiotic resistant pattern of the pathogenic and non-pathogenic isolates will be looked at the compared with the human population of the same locality. Groups of animals from Govt. farm will be compared with those from the local market.

(8) Reviews:

(a) Ethical Review Committee: \_\_\_\_\_

(b) Research Review Committee: \_\_\_\_\_

(c) Director: \_\_\_\_\_

(d) BMRC: \_\_\_\_\_

Attachment 1a

Abstract Summary:

The study will cover the isolation and characterisation of the etiologic agents responsible for causing diarrhea in domestic animals. Samples will be collected from Hospitals, markets etc. A group of normal farm animal will also be included for comparison.

Samples will be collected in sterile containers and be looked for parasites followed by culturing for pathogens using standard culture techniques.

The study will be involved any human subject and as such there is no potential risk or anything of concern to human subject.

## SECTION II - RESEARCH PLAN

### A. INTRODUCTION

1. Objectives: (1) The main objective of this study is to study thoroughly the causative agents responsible for diarrhea in domestic animal population in Bangladesh. (2) This one year study will also show the seasonal pattern of the pathogens responsible for causing diarrhea in domestic animals. (3) The antibiotic resistance pattern of the isolates from this study will help us to compare them with the pattern obtained with the isolates from human population which will establish the possible role of the plasmid transfer from one population to other.

2. Background: According to most field veterinarians of Bangladesh, the incidence of diarrheal disease in animals are very high in this country. Almost everyday, animals suffering from diarrheal diseases are brought to various Vet. Hospitals for treatment. Sometimes the nature of the disease is so acute that the animal die before treatment is instituted.

Diarrhea in animals are manifestation of several diseases. There are more than 40 pathogenic factors which cause diarrhea in cattle and poultry. Etiologic factors responsible are bacteria, parasite, virus, indigestion and other toxic mani-

Contd.

festations. The most important bacterial diseases are Salmonellosis, Staphylococcal disease, Streptococcal disease, Bovine tuberculosis, Vibrio infections, Yersiniosis and Campylobacteriosis (1) and (2). No integrated and planned work on the incidence of diarrheal disease in animals has been conducted in our country and as such there is no epidemiologic information about them. The etiologic factors are so important that no control measures could be undertaken without getting information about them. On the other hand, the chronic cases which are so common, cause a reduction in meat and milk production and affects the fertility rate in domestic animals causing considerable economic loss to the Livestock sector of Bangladesh.

Only two responsible groups of bacteria for diarrhea in cattle and poultry have been studied in Bangladesh (3). The two groups are Salmonella and E. coli. The others have not been studied in any planned basis. The two main group affected by Salmonella sp. is poultry and cattle. Studies done in several countries have shown that the host specific *S. pullorum* and *S. gallinarum* accounts for twothird of Salmonella infection in poultry and *Sal. typhisuis* accounts for a further 15-20 % . *S. dublin* is specially adapted to cattle and causes disease in calves as well as in adult animals in many part of the world.

Recent studies have shown that a small proportion of the E. coli isolated from animal diarrhea and due to ETEC (5) (6). Staphylococcal and Streptococcal infections are also common but

were not documented bacteriologically. Newer pathogens such as *Campylobacter* and *Yersinia* have been recognised in developed countries. These methods have been utilised by us for isolating these agents from human cases. The same methods can be used for *Campylobacter* and *Yersinia* from animals.

3. Rationale: The high morbidity and mortality of domestic animals due to diarrheal diseases cause a reduction in production of milk and meat which consequently will reflect indirectly the nutritional status of people. This needs a more scientific study with a view to identifying and characterizing the responsible etiologic agents on one hand, and on the other hand to establish, if any, role of the plasmid Resistant transfer from animal isolates to human isolates.

#### B. SPECIFIC AIMS

The specific aims of this study is to

- (a) Isolate and characterise the different pathogens and nonpathogens isolated from various domestic animals brought to the veterinary hospital in Dacca and Mymensingh and to compare them with a group of normal animals
- (b) to find the seasonal incidence of the etiologic agents causing diarrhea
- (c) to look at the sensitivity pattern of the various pathogens and non-pathogens to establish its role of transmission of drug resistance to human population
- (d) to look at the differential incidence of diarrheal disease in farm animals and free living domestic animals
- (e) to compare the pathogens isolated from animals with those from human population.

C. METHODS AND PROCEDURES

- (a) Collections of Specimens: Faecal samples will be collected from the animal under study. The specimen to be collected and put into a clean sterile glass container and brought back to the laboratory. Where collection of faecal sample is not possible specially prepared rectal swabs should be taken for the study. The plating should preferably be done in the field and the microscopic examination to be done in the laboratory. When facility of plating is not available in the field all steps to be done in the laboratory. It must be made clear that the specimens must reach the laboratory for processing within 3-4 hours of collection. Approximate 200 cattles, 300 poultry and 200 other domestic animal will be taken under study.

Samples will be collected from Mymensingh and Dacca (Hospital, market and Farm samples). Hospital samples will include only those which reports with diarrhea of any sort. Market samples will be sporadic and the Farm samples will be those without any known disease. A specified form which includes all the information will be made and filled up for samples to be collected for any animals.

Sampling populations:

<u>Hospital</u>	<u>Cows</u>	<u>Chicken</u>	<u>Other domestic animals</u>
Dacca	50	75	50
Mymensingh	50	75	50
Market Samples	50	75	50
Farms Sample	50	75	50

Contd.

(b) Processing of Specimens:

(i) Microscopic examination will include a search for pus cells, RBC, macrophages, ova and cysts of parasites.

(ii) Culture methods: For Vibries the specimens should be plated onto TTGA (Monsur's medium) and enriched into EP (T). The bile peptone tellurite enriched broth to be plated onto TTGA after 6-8 hours incubation.

For Salmonella, Shigella and E. coli samples to be plated onto MacConkey DCA and SS Agar and suspected colonies confirmed biochemically and serologically using standard techniques. Two single E. coli colony and a pool of 5 colonies to be picked for looking for enterotoxigenicity and enteropathogenicity.

Campylobacter sp. will be looked at by culturing onto special campy BAP medium under microaerophilic condition and confirmed biochemically and morphologically using standard procedures.

Yersinia enterocolitica will be looked at by culturing onto MacConkey SS and specially prepared Yersinia medium and enriching onto special medium in cold. Subcultures made from cold enrichment after 2-3 weeks and suspected colonies confirmed using standard methods.

For Rotavirus samples in swab should be put into PBS and kept frozen till tested using ELISA techniques. For Adenovirus a 10-15 % stool suspension to be made in PBS and the samples to be preserved in frozen conditions till a suitable method and procedures set up to test for Adenovirus.

#### D. SIGNIFICANCE

The study is of special significance as the zoonotic disease prevalent in our country has never been studied on the basis of a completed planned project. The diseases caused by various bacterial and parasitic agents are prevalent in most part of Bangladesh and causes huge economic loss to our country. More over a better understanding of the diseases in animal population will help us to study its spread in human as in most of our villages of our country the human and animal relationship is so near. Study of the antibiotic resistant pattern of the isolates will show whether the large population of bacteria isolated from human in our country have the same plasmid or R-factor as there from the animals.

#### E. FACILITIES REQUIRED

- (a) Laboratory space - Working bench space for one technician (available - no additional space needed).
- (b) Animal resources - 2400 Infant mouse to do 1200 ST assays.
- (c) Logistic support - Automobile for sample collection - 2000 miles. 20 return trips to Mymensingh by train for specimen collection.
- (d) Specialised equipment - None.

#### F. COLLABORATIVE ARRANGEMENTS

The work will be carried out in collaboration with the Bangladesh Agricultural University, Mymensingh. Dr. Bitish Chandra Devnath, DVM will be working full time as a Co-Investigator in the study. Dr. Abdur Rahman, Head, Department of Medicine and Surgery, Faculty of Vet. Science, BAU, Mymensingh will give advice on specific issues on animal disease.

## REFERENCES

1. OHC handbook series in zoonoses  
Section A: Bacterial Rickettsial and mycotic diseases, Volume I & II,  
Editor in Chief. James H. Steele.
2. Joint FAO-WHO expert committee on zoonoses, Third report. Published by  
FAO and WHO, World Health Organization, Geneva 1967.
3. Huq. M. Imdadul. Study on the bacterial Zoonoses with reference to  
Salmonellosis and colibacillosis. Manuscript presented at the WHO  
sponsored first National Seminar on Zoonoses.
4. Morne E.V. and Duncan H.A. Salmonellosis - an environmental problem  
affecting animals and man. Proc. 78th annual meeting. US animal Health  
Association. Richmond Va. 1974-288.
5. Moon H.W. Pathogenesis of Enteric disease caused by E. coli. Advances in  
Vet. Science and comparative medicine. Vol. 18, Academic Press, New York.  
1974, 179.
6. Ellis R.P. and Kienholz J.C. Heat labile enterotoxin produced by Escherichia  
coli serogroup O149 isolated from diarrhetic calves, Infect. Immun. 1,  
1002, 1977.

SECTION III - BUDGET

A. DETAILED BUDGET

1. PERSONNEL SERVICES

<u>Name</u>	<u>Position</u>	<u>Percent of effort or number of days</u>	<u>Annual Salary</u>	<u>Project requirement Taka</u>	<u>Dollar</u>
M.I. Huq	Branch Head	15 %	30000		4500
N.C.Devnath	Co-Investigator	100 %	-	-	
Abdul Haque	Res. Technician	50 %	32100	16,050	
New	Lab. Technician	100 %	16800	16,800	
Z.A. Khan	Lab. Attendant	50 %	14796	7,398	
Per Diem for travel to and from Mymensingh				8,000	
				48,248	

2. SUPPLIES & MATERIALS

700 stool samples for Vibrio Sal, Shig. @ 15.50		10,850
700 stool for Campy & Yersinia @ 12.50		8,750
1200 LT testing : 1200 ST testing :	@ 14.50	17,400
1000 sensitivity testing @ 9.00		9,000
700 Microscopic examination @ 2.05		1,435
Office supplies		2,000
Miscellaneous supplies		2,000

3. EQUIPMENT

No extra equipment needed.

4. PATIENT HOSPITALIZATION

None

5. OUTPATIENT CARE  
None
6. ICDDR,B TRANSPORT  
2000 miles automobile transport @ 3.50 per mile Tk. 7,000.00
7. TRAVEL & TRANSPORTATION OF PERSONS  
Travel (train) two persons 12 times Tk. 2,400.00
8. TRANSPORTATION OF THINGS  
Media, Plates, Samples Tk. 3,000.00
9. RENT COMMUNICATION & UTILITIES  
None
10. PRINTING & REPRODUCTION  
Xerox Tk. 1,000.00  
Others Tk. 1,000.00  
Publication Tk. 2,000.00
11. OTHER CONTRACTUAL SERVICES  
None
12. CONSTRUCTION, RENOVATION, ALTERATIONS  
None

BUDGET SUMMARY

	<u>Taka</u>	<u>Dollar</u>
1. Personnel	48,248.00	4,500.00
2. Supplies	51,435.00	-
3. Equipment	-	-
4. Hospitalisation	-	-
5. Outpatient care	-	-
6. ICDDR,B transport	7,000.00	-
7. Travel	2,400.00	-
8. Transport, thing	3,000.00	-
9. Rent/Communication	-	-
10. Printing/Publication	4,000.00	-
11. Contractual Services	-	-
12. Constructions	-	-
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Sub-total	116,083.00	4,500.00
	= US \$ 7,738.00	
Total US \$ 7,738.00 + 4,500.00 = US \$ 12,238.00		
30 % Overhead US \$ 3,671.00		
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Grand Total	US \$ 15,909.00	