BACTERIAL LOAD IN THE FECES OF RABBIT AT DINAJPUR DISTRICT OF BANGLADESH

A THESIS

BY

MITA RANI DAS

Registration No.: 1505006

Semester: January-June, 2017

MASTER OF SCIENCE (M.S.)

IN

MICROBIOLOGY

DEPARTMENT OF MICROBIOLOGY
FACULTY OF POST GRADUATE STUDIES
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

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Department of Microbiology
Faculty of Post Graduate Studies
Hajee Mohammad Danesh Science and Technology University,
Dinajpur, In partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE (M.S.)
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DEPARTMENT OF MICROBIOLOGY
FACULTY OF POST GRADUATE STUDIESHAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

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Dedicated
To
My Beloved Parents

ACKNOWLEDGEMENTS

At first all praises and deepest sense of gratitude be to Almighty, the supreme creator of at first all praises and deepest sense of gratitude be to Almighty, the supreme creator of this universe, who enabled the author to complete this thesis.

The author would like to express her deepest sense of gratitude, sincere appreciation, profound regards and indebtedness to her reverend teacher and research supervisor, ***Dr. Farzana Afroz,*** *Assistant Professor, Department of Microbiology of Veterinary and Animal Science, , Hajee Mohammad Danesh Science and Technology University, Dinajpur, for her scholastic and dynamic guidance, constant inspiration, cordial consistence, affectionate feeling, utmost desire, sympathetic supervision and constructive criticism in all phases of this study and preparing of the manuscript.*

The author finds it a great pleasure in expressing her heartfelt gratitude and immense indebtedness to her honorable and respected teacher and research co-supervisor, ***Dr. Mir Rowshan Akter,*** *Associate Professor, Department of Microbiology, HSTU, Dinajpur, for her constant help, cooperation and inspiration to conduct the course work and research smoothly and perfectly.*

The author humbly desires to express her profound respect and sincere appreciation to her respectable teacher Dr. Md. Khalid Hossain, Associate Professor and Chairman, Dr. Md. Atiqul Haque, Dr. Nazmi Ara Rumi, Dr. Md. Khalesur Rahman and Delowara Begum, Lecturer, Department of Microbiology, HSTU, Dinajpur, for their constant help, cooperation and inspiration to conduct the course work and research smoothly and perfectly.

The author express her thanks to Md. A. Ayub, Technician, Department of Microbiology, HSTU, Dinajpur for their sincere cooperation towards the completion of the research work.

Finally, the author is ever indebted to his beloved parents, brothers and sisters for their endless sacrifices, heartiest blessings and out support throughout his entire life.

The Author

June, 2017

ABSTRACT

The present study was conducted to identify the bacterial load in feces of rabbit and to identify their effects on growth performance of rabbit of Dinajpur Upazilla, Dinajpur. The samples were analyzed to determine the total viable count, prevalence of gram negative bacteria, *Eschrechia coli* and *Salmonella spp*. The rabbit were categorized into three (3) groups namely group A, group B and group C according to their body weight gain at same age and different feeding management. They reared in intensive system. For the determination of bacterial load a total of 30 samples were tested. From this study the result represented that the total viable counts/g feces in group A, group B and group C were 1.2×10^4, 2.4×10^6, 3.5×10^7 CFU/g sample respectively, the *E. coli* counts/g feces in group A, group B and group C were 2.5×10^3, 5.5×10^5 and 4.5×10^6 CFU/g of sample respectively and Salmonella spp. counts/g sample on group A, group B and group C were 1×10^2, 2.1×10^2 and 3×10^3 CFU/g feces respectively. The study showed that group C had highest bacterial load 3.5×10^7 CFU/g (7.54 Log 10/g) and average body weight these group rabbits 1 kg. In group A had lowest bacterial load 1.2×10^4 CFU/g sample (4.08 Log10/g) and average body weight these group rabbits 1.5 kg. where the prevalence of *E. coli* present in 13 sample was 43.33%, group A- 30%, Group B -40% and group- C 60%, and the prevalence of *Salmonella spp* was present in 7 samples was 23.33%, group A- 10%, Group B- 20% and group C-40%. In conclusion, it is evident that *E. coli* and *Salmonella spp* was successfully detected through different bacteriological media and biochemical reaction indicating the isolated organisms were responsible for possible fecal pollution and also a negative effect on growth performance of rabbit reared in research area.

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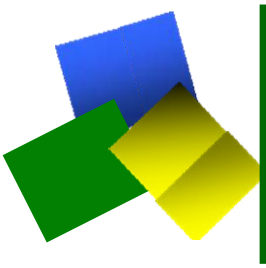
LIST OF ABBREVIATION AND SYMBOLS

TVC	:	Total viable count
TEC	:	Total *E. coli* count
CFU	:	Colony forming units
cm^2	:	Centimeter square
E. Coli	:	*Escherichia coli*
e.g.	:	Example
ELISA	:	Enzyme-linked immunosorbent assay
EMB	:	Eosin Methylene Blue agar
et al.	:	Associated
TSC	:	Total *Salmonella spp* count
EU	:	European Union
FAO	:	Food and Agriculture organization
Fig.	:	Figure
GDP	:	Gross Domestic Product
GNP	:	Gross National Product
Gm	:	Grams
hrs	:	Hours
HSTU	:	Hajee Mohammad Danesh Science and Technology University
H_2S	:	Hydrogen sulphide
HWS	:	Hot Water Supply
ISO	:	International Organization for Standardization
Ib	:	Pound
Kg	:	Kilogram
log	:	Logarithms
MCA	:	MacConkey Agar
mg	:	Milligram
min	:	Minutes
MIU	:	Motility Indole Urease
ml	:	Milliliter
mm	:	Millimeter
NA	:	Nutrient agar
No.	:	Number

PCR	:	Polymerase Chain Reaction
PH	:	Numerically it is the negative logarithm of that concentration of hydrogens ions (= H+) (= protons) in a solution.
Prof.	:	Professor
SL.	:	Serial
SPC	:	standard plate count
spp.	:	Species
Sq	:	Square
SSA	:	Salmonella-Shigella agar
TBC	:	Total bacterial counts
TCC	:	Total coliform count
TSI	:	Triple Sugar Iron agar
TVC	:	Total viable counts
WB	:	Warner-Bratzler
US	:	United State
WHO	:	World Health organization
-	:	Negative
%	:	Percentage
/	:	Per
+	:	Positive
<	:	Less than
>	:	Greater than
µg	:	Microgram
^{0}C	:	Degree Celsius



CHAPTER I

INTRODUCTION

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INTRODUCTION

Bangladesh is an agro-based country where 80 percent of the population live on agriculture. Since livestock plays a vital role in the economy of Bangladesh, therefore its contribution bears significant strain on maintaining domestic resources essential for promotion of lifestyle and livelihood of landless and marginal farmers. It is evident that livestock wealth provides about 9% of the Gross National Product (GNP), which is 6.5% of Gross Domestic Product (GDP) (Ahmed, 1992; Rahman and Rahman, 1998).

Micro-livestock like rabbit can play a significant role for poverty alleviation among smallholder farming community in Bangladesh. They are found in different districts and almost all of them are mainly reared by the landless and marginal farmers in the rural areas and very few portions of their population are reared by the inhabitant of metropolitan cities.

Rabbits in Bangladesh are scattered and distributed all over the country. However, the rabbits are found in a great number in the region of Muktagacha, Haluaghat, Modhupur, Tangail and in some parts of Dhaka. In commercial form most of the rabbits are found in various research organization, laboratories, and educational institutions. Commercial farming of rabbit in Bangladesh is still very limited although there is a demand of this animal. In most cases the research institute is rearing rabbit for research purposes under confinements. In the rural areas, where the people rear it in large or medium scale, follow semi-intensive system, some times intensive system as well (Khatun *et al.,* 2016).

Now a days, many NGO's and other Govt. organizations have taken initiative on rabbits farming as an income generating activity for the destitute women and school going children. Farmers rearing small scale rabbit opined that rabbits farming is obviously profitable enterprise but sometimes marketing problem of rabbit have occurred those who reside in the remote areas. But the farmer resides in the metropolitan cities or town did not face such type of marketing problems. People resides in the metropolitan cities purchased rabbit for meat consumption as well as game animal. Rabbit could be reared in homestead with minimum cost by proper utilization of family labour and homestead feed sources with little or no extra inputs without hampering the other farming activities. Rabbit feed voraciously on kitchen waste which is an added advantage in its feeding

habit. Being a non-ruminant herbivore, rabbits are endowed with large hindgut that facilitates efficient digestion of feed particularly forages because of the heavy presence of microorganisms. It is therefore considered as a hindgut fermentor. The hindgut consist of the caecum and colon and a large intestine heavily loaded with microorganism enabling the rabbits to utilise fibrous feed efficiently by courtesy of its feeding and digesting strategy (Iribeekel, 2001).

The domestic rabbit being primarily herbivorous, feeds on most green vegetables, hay, grains, tuber and roots thriving excellently on rations with ingredients almost entirely fromplant sources (Aduku and Olukosi, 1990; Nodu *et al.,* 1999; Oruseibio, 2002). It has not been received due attention though it is a source of good quality meat. The meat of rabbit is a rich source of protein, energy, minerals and vitamins and low in fat, sodium and cholesterol. It is also reported that rabbit meat is delicious and higher in protein contents. (Jones, 1990; Handa *et al.*1995; Ali and Sukanta,1993; and Sandford,1986).

Rabbit meat is well known for its high protein content serving of rabbit meat contains 28 g of protein, more than beef or chicken. Rabbit is also a concentrated source of iron. A serving contains more than 4 mg. Additionally, the meat provides a wide range of minerals. The highest levels include 204 mg of phosphorous and 292 mg of potassium. The calories in rabbit meat are low. A serving contains only 147 calories. (FAO - The Rabbit - Husbandry, health and production).

In order to maximize the food production in Bangladesh, all possible options including rabbit production may be addressed. The climatic conditions, commercial factors, ecological environment, religious points of view, social practices and technological know-how support the rabbit production in Bangladesh (MIDAS, 1992).

There are many potential benefits that may be realized from backyard rabbit raising. When planning rabbit enterprise it will be important to dentify a goal and anticipated benefits from farming rabbits. An example of a primary goal is to produce enough rabbits so that the family might consume meat from two fryers per week the year-round. This production goal would require a small backyard operation consisting of about four breeding females (does) and one breeding male (buck). As a backyard activity, and relative to most livestock or outdoor pet animals, rabbits are quiet, odorless and docile animals that often go unnoticed by neighbors, even in residential areas. However, residents living within legal city limits should inquire about possible restrictions

concerning the keeping of rabbits by c ontacting their county health department office. (Lukefahr *et al.,* 1998).

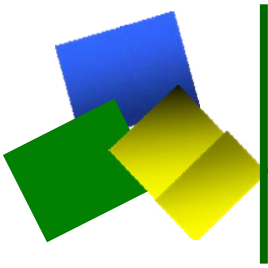
Some strains of *E. coli* possess pathogenic *Escherichia coli* is considered as the normal bowel flora of different species character due to the acquisition of virulent factors. Microbial characteristics associated with virulent *E. coli* include production of enterotoxin, verotoxin, colicins and siderophores, type-1 pili and motility, resistance to the lytic action of the host complement and antibiotics (Dho and Lafont, 1984; Chulasiri *et al.,* 1989).

Salmonellosis has been recognised in all countries, but appears to be most prevalent in areas of intensive animal husbandry, especially in pigs and calves and some types of poultry reared in confinement. Reptiles are commonly asymptomatic carriers of *Salmonella*. Several serovars are host specific (e.g. *S. abortusovis* in rabbit or *S. Typhi* in humans) or host adapted (e.g. S. *Choleraesuis, S. Dublin*). (OIE, 2008).

Salmonellosis in animals intended for human consumption is of great concern from the Public Health perspective (EC, 2003). Monitoring and surveillance protocols of the rabbit farms include this disease and its risk factors, in particular on farms in the open air. From the perspective of Associations for managing Animal Health in food-producing animals protocols include the comparison of strains between affected farms and its connections with centers of selection, breeder's multiplication or insemination centers, as well as its relationships with slaughterhouse. (Saco *et al.,* 1997).

The present study was conducted with the following objectives-

- To determine the bacterial load in feces of rabbit.
- To isolate the bacteria from rabbit feces.
- To know the effects of isolated bacteria on growth performance of rabbit.



CHAPTER II

REVIEW OF LITERATURE

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REVIEW OF LITERATURE

Medubari *et al.* (2016) Studied that, rabbit is an herbivorous animal habitually practicing caprophagy which synergistically works along with the large bacteria population in the gut to efficiently digest roughages and other plant materials especially green vegetables. Results showed that total heterotrophic bacteria (THB) counts and coliforms were respectively recorded in the range of 1793 – 2720 CFU/g and 111.67 – 189.00 cfu/g.

Atwill *et al.* (2015) studied that, a field trial in Salinas Valley, California, was conducted during July 2011 to quantify the microbial load that transfers from wildlife feces onto nearby lettuce during foliar irrigation. Romaine lettuce was grown using standard commercial practices and irrigated using an impact sprinkler design. Five grams of rabbit feces was spiked with 1.29×10^8 CFU of Escherichia coli O157:H7 and placed - 3, - 2, and - 1 days and immediately before a 2-h irrigation event. Immediately after irrigation, 168 heads of lettuce ranging from ca. 23 to 69 cm (from 9 to 27 in.) from the fecal deposits were collected, and the concentration of E. coli O157:H7 was determined. Thirty-eight percent of the collected lettuce heads had detectable E. coli O157:H7, ranging from 1 MPN to 2.30×10^5 MPN per head and a mean concentration of 7.37×10^3 MPN per head. Based on this weighted arithmetic mean concentration of 7.37×10^3 MPN of bacteria per positive head, only 0.00573% of the original 5 g of scat with its mean load of 1.29×10^8 CFU was transferred to the positive heads of lettuce. Bacterial contamination was limited to the outer leaves of lettuce.

Borrelli *et al.* (2010) Observed that, a total of 1,000 rectal samples were collected from rabbits coming from 25 rabbit farms in southern Italy. All samples were processed for isolation of Salmonella spp. by standard culture method based on the ISO 6579:2002 method. *Salmonella spp*. was isolated from 1/25 rabbit farms analyzed. In particular, four out of 1,000 rectal swab samples, taken from young rabbits, were serotyped as *S. Typhimurium* and phage typed as *S. Typhimurium* DT104.

Gallois *et al.* (2007) Mentioned that rabbit sludge had the highest fecal *coliform* (CFC) contamination with a mean density of 1.5×10^2 MPN/100 ml and *Vibrio* and *Salmonella*

species were more frequently detected in samples which also showed high incidence of fecal bacteria.

Maria Magliulo *et al.* (2007) studied that total viable count from rabbit feaces TVC 10^4–10^5 CFU/gm of feaces.

Gourmelon *et al.* (2007) identified the origin of the fecal contamination observed in French estuaries, two library-independent microbial source tracking (MST) methods were selected: i) Bacteroidales host-specific 16S rRNA gene markers and ii) F-specific RNA Bacteriophage genotyping. The specificity of the Bacteroidales makers was evaluated on human and animal (bovine, pig, sheep and rabbit) feces.

Graves *et al.* (2002) reported the general methods of isolates from known sources is to collect fecal samples directly from the animals or from fresh droppings.

Graziani *et al.* (2004) reported 1908 isolates of *S. Typhimurium* from human (n=755), bovine (n=122), swine (n=632), chicken (n 10 =94), turkey (n=117), rabbit (n=83), and pigeon (n=105); among them 242 strains were phagetyping as *S. Typhimurium* DT104. In southern Italy, although all isolates were phagetyped as *S. Typhimurium* DT104, *S. Typhimurium* is scarce in rabbits. *S. Typhimurium* can cause severe enteritis with high mortality percentages in fattening rabbits; in doe rabbits *S. Typhimurium* produces enteritis and metritis usually associated with abortions and heavy losses inside the nests .In suckling rabbits *S. Typhimurium* can cause diarrhea, haemorrhagic enteritis, splenomegalia, and fibrinous peritonitis. In the present study, however, all Salmonella positive rabbits were in healthy conditions although one rabbit showed diarrhea. The environmental samples were negative except for the nest boxes housing the positive rabbits.

Agnoletti *et al.* (1999) reported that, the isolation of Salmonella spp. from commercial is rabbit considered to be sporadic and, usually, of scarce pathological relevance. Nevertheless, having several outbreaks being reported in rabbit breeding farms in the north-eastern part of Italy in 1997, we decided to investigate the infection prevalence. Approximately 2700 fecal and environmental swabs were collected in 23 commercial rabbitries, and in each farm feeding stuffs were also sampled. Samples were individually processed by traditional cultural methods. Salmonella spp. was isolated from 7 farms (30.4 per cent), whereas all feed samples were negative. Serotyping of isolates identified

S. Indiana (6 units) and *S. Typhimurium* (1 unit). In the rabbitry where *S. Typhimurium* was widespread economic losses were high, due to haemorrhagic necrotic metritis in does and to septicemia and haemorrhagic enteritis in suckling animals. S. Indiana did not appear to affect rabbits health condition. Results pointed out the high percentage of salmonella free commercial rabbitries but emphasized the prevalence of S. Indiana infection this serotype represents a possible source of meat contamination at the slaughterhouse, although not influencing animal productive and sanitary parameters. *S. Typhimurium* instead appeared as highly pathogen for rabbits. Moreover, due to its multidrug resistance pattern, it represents an hazard for human health and its low prevalence allows to consider the possibility of eradicating the infection rather than controlling it.

Laura Tessmer *et al.* (1998) reported that, several conditions such as stress; a high fat, low fiber diet; too many carbohydrates, etc are the factor for growing of opportunities bad bacteria. These bad bacteria produce toxins that can be harmful or fatal to your rabbit. On the other hand, the products of good cecal fermentation are crucial to healthy gut flora, because through coprophagy, the oral re-ingestion of the cecal pellets produced by this fermentation process, the rabbit can absorb by normal digestion the special nutrients and vitamins contained in the cecal pellets. Some evidence suggests that bacteria from these (re-ingested) cecal pellets help the food digest while in the stomach.

Foster *et al.* (1997) found that, in rabbits, an abundant microbiota (1010 to 1012 bacteria /gm) is present forming unit) of cellulolytic bacteria per gram of feces.

Saco *et al.* (1997) evaluated that, the incidence risk of salmonellosis on 394 commercial rabbit farms in Spain (374) and Portugal (21). Data were gathered on 2,269 visits performed by a trained veterinarian, during 2008-2011. The median size of the farms was 740 does (minimum to maximum: 40-9,000 does), and 50 bucks (minimum to maximum: 8-544 bucks). Refrigerated samples obtained from on- farm necropsies of aborted does, kits and runt growing rabbits were analyzed for isolation and typing of Salmonella spp. Clinical incidence risk was 4%, the same result as in a precedent retrospective study performed by the same practitioner, on 868 rabbit farms during 1997-2007. From the perspective of the Associations for managing Animal Health in food-producing animals, it was useful to point out the diffusion of infected young breeders and semen from

selection breeder's centers and artificial insemination centers, respectively, to production farms

Lea and Febinger (1995) mentioned that, quick changes to the diet can cause diarrhea or an overgrowth of bad bacteria in the gut of rabbit. Also it should be kept in mind that different rabbits have different dietary needs. One thing to avoid is sugar; as it increases the bad bacteria in their intestines and can cause disease resulting in diarrhea and loss of appetite. Also spinach, cabbage, cauliflower, mustard, raw beans, potato peals, rhubarb, or any spoiled food should be avoided.

Fonty *et al.* (1989) the total viable counts and selective enumeration of anaerobic bacteria in the caecal content, soft and hard faeces of rabbit. Ecological factors determining establishment of cellulolytic bacteria.

Sorlini *et al.* (1988) found that, Total viable counts in rabbit feaces TVC 10^2–10^3 CFU/gm of feaces.

Khaw *et al.* (1993) evaluated bacterial infections may include the *E. coli* bacteria or a disease known as coccidiosis, caused by the coccidian bacteria. Generally it is an imbalance of the good and bad bacteria within the rabbit's gut which result in diarrhoea. The rabbit's digestive system relies heavily on the correct balance of the different types of bacteria.

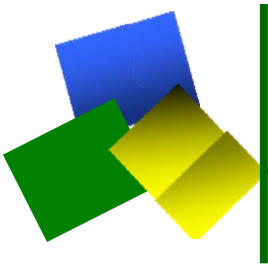
Dana Krempels *et al.* (1998) showed that, rabbits have several kinds of bacteria living in their digestive track (so do all animals), a good kind that helps them to break down food an absorb it and some bad kinds like E-coli that all living animals have. When something happens to cause the food to ferment in the rabbits stomach the bad kind of bacteria level become too high and will make the rabbit very sick.

Emaldi *et al.* (1976) studied that, total viable counts, proteolytic, ureolytic, cellulolytic and NH^+_4 utilizing anaerobic bacteria in caecal contents, soft and hard faeces of rabbits were studied. No significant differences between the bacterial counts from the caecum and soft faeces were observed ($p < 0{\cdot}05$) but there were significant differences between the bacterial counts of the soft and hard ($p < 0{\cdot}01$).

Aruna *et al.* (2010) evaluated that, enterohemorrhagic *Escherichia coli* (EHEC) produce one or more types of Shiga toxins and are foodborne causes of bloody diarrhea. The

prototype EHEC strain, *Escherichia coli* O157:H7, is responsible for both sporadic cases and serious outbreaks worldwide. Infection with *E. coli* that produce Shiga toxins may lead to diarrhea, hemorrhagic colitis, or (less frequently) hemolytic uremic syndrome, which can cause acute kidney failure. The exact mechanism by which EHEC evokes intestinal and renal disease has not yet been determined. The development of a readily reproducible animal oral-infection model with which to evaluate the full pathogenic potential of *E. coli* O157:H7 and assess the efficacy of therapeutics and vaccines remains a research priority. Dutch belted (DB) rabbits are reported to be susceptible to both natural and experimental EHEC-induced disease, and New Zealand white (NZW) rabbits are a model for the intestinal manifestations of EHEC infection. In the current study, we compared the pathology caused by *E. coli* O157:H7 infection in DB and NZW rabbits. Both breeds of rabbits developed clinical signs of disease and intestinal lesions after experimental infection. In addition, one of the infected DB rabbits developed renal lesions. Our findings provide evidence that both breeds are susceptible to *E. coli* O157:H7 infection and that both may be useful models for investigating EHEC infections of humans.

Alton *et al.* (2012) reported that, enteropathogenic Escherichia coli (EPEC) is the most important cause of persistent diarrhea in children, particularly in developing countries. Animals serve as pathogenic E. coli reservoirs, and compelling evidence for cross-species EPEC transmission exists. In this report, enzootic EPEC infection associated with up to 10.5% diarrhea-associated morbidity in a large laboratory Dutch Belted rabbit colony was investigated. These rabbits were obtained from a commercial vendor and had acute diarrhea following shipment. Fecal culture of 20 rabbits yielded 48 E. coli isolates, 83% of which were *eae* positive. Repetitive sequence-based PCR (REP-PCR) and serologic analysis identified a single disease-associated EPEC O145:H2 strain. In sampled rabbits, EPEC-positive culture and the presence of diarrhea were significantly associated. This strain displayed a localized adherence-like HEp-2 cell adherence pattern, as seen in diarrheic human infant EPEC isolates. Treatment was instituted with the fluoroquinolone antibiotic enrofloxacin, to which all isolates were susceptible. Preshipment parenteral enrofloxacin administration reduced diarrhea-associated morbidity 22-fold and mortality 12-fold in subsequent deliveries. This report emphasizes the zoonotic potential of animal EPEC strains and the need for virulence determinant-based screening of E. coli isolates from diarrheic animals.



CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

The present research work was conducted during January to June, 2016 at the Microbiology laboratory in the Department of Microbiology, Hajee Mohammad Danesh Science and Technology University, Dinajpur. The detailed outline of Materials and Methods are given below.

3.1 Materials

3.1.1 Study area

The research work is carried out at three selected area of Dinajpur district of Bangladesh. Namely HSTU Rabbit Farm Basherhat, Doshmile and Koshba.

3.1.2 Study population

A total 30 rabbits were categories into three different groups on the basis of body weight age and feeding namely group A, group B and group C. All rabbits belongs to three groups were reared in intensive system.

3.1.2.1 Group A

Ten animals were selected randomly for this study of 4 months aged having average 1.5 kg body weight. They were fed with napier grass and pellets.

3.1.2.2 Group B

Ten animals were selected randomly for this study of 4 months aged having 1.2kg body weight. They were fed with napier grass and vegetables (pototos, carrot etc.).

3.1.2.3 Group C

Ten animals were selected randomly for this study of 4 months aged having 1kg body weight. They were fed with only of vegetables (pototos, carrots, cabages etc.).

3.1.3 Field sample

Fecal samples of rabbit were collected aseptically directly from the rectum, just after deposition and taken into small polybags using disposable hand gloves. After collection

all the samples were brought to the Department of Microbiology, Hajee Mohammad Danesh Science and Technology University for laboratory analysis maintaining proper cool chain.

3.1.4 Instrument and appliances

Phase contrast microscope, test tubes, cotton, hand gloves, plastic syringe (5 ml), micropipette (1 ml, 500 µl, 10-20 µl), glass slides, eppendorf tubes, magnifying glass, marker pen, ice-box, spirit lamp, cover slips, inoculating loop and rack, autoclave, refrigerator, conical flask, colony count machine, digital weight balance and stirring machine

3.1.5 Laboratory preparation

All items of glasswares including test tubes, pipettes, cylinder, flasks, conical flasks, glass plate, slides, vials and mesearing cylinder soaked in a household dishwashing detergent solution ('Trix, Recket and Colman Bangladesh Ltd.) for overnight, contaminated glasswares were disinfected in 2% sodium hypo chloride solution prior to cleaning. The glassware were then cleaned by brushing, washed thoroughly and finally sterilized by autoclaving for 15 minutes at 121^0 C under 15 lbs pressure per square inch. Autoclaved items were dried in a hot air oven over at 50^0 C. Disposable plastic were (micropipette tips) was sterilized by autoclaving. All the glassware was kept in oven at 50^0 C for future use.

3.1.6 Different Media that were used

3.1.6.1 Media for cultural test

3.1.6.1.1 Liquid media

1. Nutrient broth

Nutrient broth (NB) was used to grow the organisms from the samples collected from the study areas before performing biochemical test (Cheesebrough, 1985).

13 gram of Bactonutrient broth (Difco) was dissolved in 1000 ml of cold distilled water and heated upto boiling to dissolve it completely. The solution was then distributed in tubes, stoppered with cotton plugs and sterilized in the autoclave machine at l21°C and 15 pounds pressure per square inch for 15 minutes. The sterility of the medium was

judged by incubating overnight at 37°C and used for cultural characterization or stored at 4°C in refrigerator for future use (Carter, 1979).

2. 2% buffered peptone water (Hi-media, India).

3.1.6.1.2 Solid media

1. Nutrient agar base (Hi-media, India).

The media was prepared as per direction of manufacturer and cultivated in bacteriological Incubator following the method of Carter, 1979.

28 gram of nutrient agar powder (Hi-media, India) was suspended in 1000 ml of cold distilled water in a flask and heated to boiling for dissolving the medium completely. The medium was then sterilized by autoclaving. After autoclaving, the medium was poured into each sterile petridish and allowed to solidify. After solidification of the medium in the petridishes, these were incubated at 37^0C for overnight to check their sterility and used for culture characterization (Carter, 1979).

2. Plate count agar media (Hi-media, India).

Plate count agar was used to determine of plate count of microorganisms by pour plate method (Cheesebrough, 1985).

17.6 gram of plate count agar powder was suspended in 1000 ml of cold distilled water in a flask and heated to boiling for dissolving the medium completely. The medium was then sterilized by autoclaving. After autoclaving, 20 ml 0f the medium was poured into each sterile petridish and allowed to solidify. After solidification of the medium in the petridishes, these were incubated at 37^0C for overnight to check their sterility and used for culture characterization (Carter, 1979).

3. Salmonenella-Shigella agar (Hi-media, India).

Salmonella-Shigella (SS) agar medium was used as a selective medium for salmonella organism which causes enhancement of the grouth of salmonella while inhibiting the growth of other contaminating organisms shows typical colony character (Cheesebrough, 1985).

6.3 gram of dehydrated salmonella-shigella agar (High Media, India) was suspended in 100 ml of cold distilled water taken in a conical flask and heated upto boiling to dissolve the medium completely. After sterilization by autoclaving, the medium was poured in 10 ml quantities in sterile glass ptridishes (medium sized) and 15 ml quanties in sterile glass petrdishes (large size) to form a thick layer therein. To accomplished the surface be quite dry, the medium was allowed to solidify for about 2 hours with the covers of the petridishes partially removed. The sterility of the medium was judged by keepig the petridishes in the incubator at 37^0C for overnight and then used for cultural characterization or stored at 4^0C in refrigerator for future used (Carter, 1979).

4. Brilliant green agar (Oxoid, England).

Brilliant green agar (BGA) was used as a selective madium for the isolation and identification of *Salmonella* organism (Cheesebrough, 1985).

BGA medium was used as a selective medium for the isolation and identification of salmonella organisms (Cheesebrough, 1985). According to the direction of manufacturer 52 gm of BGA powder (Oxoid, England) was suspended in 1000 ml of distilled water in a conical flask. It was then gently heated with gentle agitation and brought just to the boil to dissolve the medium completely. After strelization by autoclaving the medium was cooled to 50°C, mixed properly and poured into sterile petridishes (10ml in each petridish) and allowed to solidify. Then the petridishes were incubated at 37°C for over night to check their sterility and used to culture the organism or stored at 4ºC in refrigerator for future use (Carter, 1979).

5. Eosin methylene blue (EMB) agar (Hi-media, India)

Eosin methylene blue (EMB) agar medium was used to observe the growth of Escherichia coli (Cheesebrough, 1985).

36 gram of EMB agar base (Hi-media, India) was added to 1000 ml of distilled water in a conical flask and heated until boiling to dissolve the medium completely. After sterilization by autoclaving, the medium was poured in 10 ml quantities in sterile glass petridishes (medium sized) and in 15 ml quantities in sterile glass petridishes (large sized) to form a thick layer therein. To accomplish the surface be quite dry, the medium was allowed to solidify for about 2 hours with the covers of the petridishes partially removed.

The sterility of the medium was judged and used or stored at 4°C in refrigerator for future use (Carter, 1979).

6. MacConkey agar medium (Hi-media, India).

MacConkey agar (MC) medium was used to culture the organisms under the family Enterobacteriaceae (Cheesebrough, 1985).

51.50 gram of dehydrated Bacto-MacConkey agar (Difco) was suspended in 1000 ml of cold distilled water taken in a conical flask and was heated up to boiling to dissolve the medium completely. After sterilization by autoclaving, the medium was poured in 10 ml quantities in sterile glass petridishes (medium sized) and in 15 ml quantities in sterile glass petridishes (large sized) to form a thick layer therein. To accomplish the surface be quite dry, the medium was allowed to solidify for about 2 hours with the covers of the petridishes partially removed. The sterility of the medium was judged and used for cultural characterization or stored at 4°C in refrigerator for future use (Carter, 1979).

3.1.6.1.3 Media used for Biochemical test

1. Triple sugar iron (TSI) agar slant (Hi-media, India).

Triple sugar iron (TSI) agar slant After isolation on the selective media, differential-screening media such as triple sugar iron agar was used to further categorized organisms. Colonies were inoculated by stab or streak into triple sugar iron (TSI) agar slants. In TSI agar, if the organisms ferment only glucose the tube will turn yellow in a few hours. The bacteria quickly exhausted the limited supply of glucose and start oxidizing amino acids for energy, giving of ammonia as an end product. Oxidation of amino acids increases the P^H and the indicator in the slanted portion of the tube will turn black to red. The butt will remain yellow. If the organism in the TSI slant farments lactose and/or sucrose, the butt and the slant will turn yellow and remain yellow for days due to the increased level of acid production. Gas production of the organisms can be ascertained by the appearance of the bubbles in the agar. TSI can also be used to indicate whether hydrogen sulphide (H_2S) has been produced due to reduction of sulphur containing compounds. H_2S reacts with the ferrus sulphate of the medium producing ferric sulphide, a black precipitateant (Hi-media, India).

2. Motility, Indole, Urease (MIU) medium (Hi-media, India).

18 grams of MIU agar (Difco) was suspended in 950 ml of cold distilled water taken in a conical flask and heated up to boiling to dissolve the medium completely. 95 ml of it was despensed into flasks and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Then it was cooled to about 50°C and 5 ml sterile 40% basal medium was added aseptically. After mixing were dispensed into sterile test tubes. Then allowed to cool in an upright position. The sterility of the medium was judged and used for cultural characterization or stored at 4°C in refrigerator for future use (Carter, 1979).

3. Methyl Red-Voges Proskaure broth

A quantity of 3.4 grams of Bacto MR-VP medium was dissolved in 250 ml of distilled water dispensed in 2 ml amount in each test tube and then the test tubes were autoclaved. After autoclaving, the tubes containing medium were incubated at 37°C for overnight to check their sterility and used for biochemical characterization or stored at 4°C in refrigerator for future use (Cheesbrough, 1985).

3.1.7 Reagents

1. Gram's staining reagent: Crystal violet, Gram's iodine, Acetone and Safranine.
2. Alpha-naphthol solution.
3. Kovac's reagent.

- **Methyl red solution**

  The indicator methyl red (MR) solution was prepared by dissolving 0.1 gm of Bacto methyl red (Difco) in 300 ml of 95% alcohol and diluting this to 500 ml with the addition of 200 ml of distilled water.

- **Kovac's reagent**

  This solution was prepared by mixing 25 ml of concentrated Hydrochloric acid in 75 ml of amyl alcohol and to this mixture 5 grams of paradimethyl-aminohenzyldehide crystals were added. This was then kept in a flask equipped with rubber cork for future use (Merchant and Packer, 1969).

- **Phosphate buffered saline solution**

  For preparation of Phosphate buffered saline (PBS) solution, 8 gram of sodium chloride (NaCl), 2.89 gram of disodium hydrogen phosphate (Na_2HPO_4, $12H_2O$),

0.2 gram of potassium chloride (KCl) and 0.2 gram of potassium hydrogen phosphate were suspended in 1000 ml of distilled water. The solution was heated to dissolve completely. The solution was then sterilized by autoclave at 121°C maintaining a pressure of 15 pounds per square inch for 15 minutes and stored at refrigerator until use. The pH of the solution was measured by a pH meter and maintained at 7.0-7.2 (Cheesbrough, 1985).

4. Ethyl alcohol (70% and 95%).

3.2 Methods

3.2.1 Experimental layout

The samples were collected directly from rabbit from Dinajpur upazilla for determination of bacterial load, isolation and identification of bacterial pathogens by morphology, staining and cultural characteristics. Characterization of bacteria were done by cultural and biochemical reactions.

EXPERIMENTAL LAYOUT

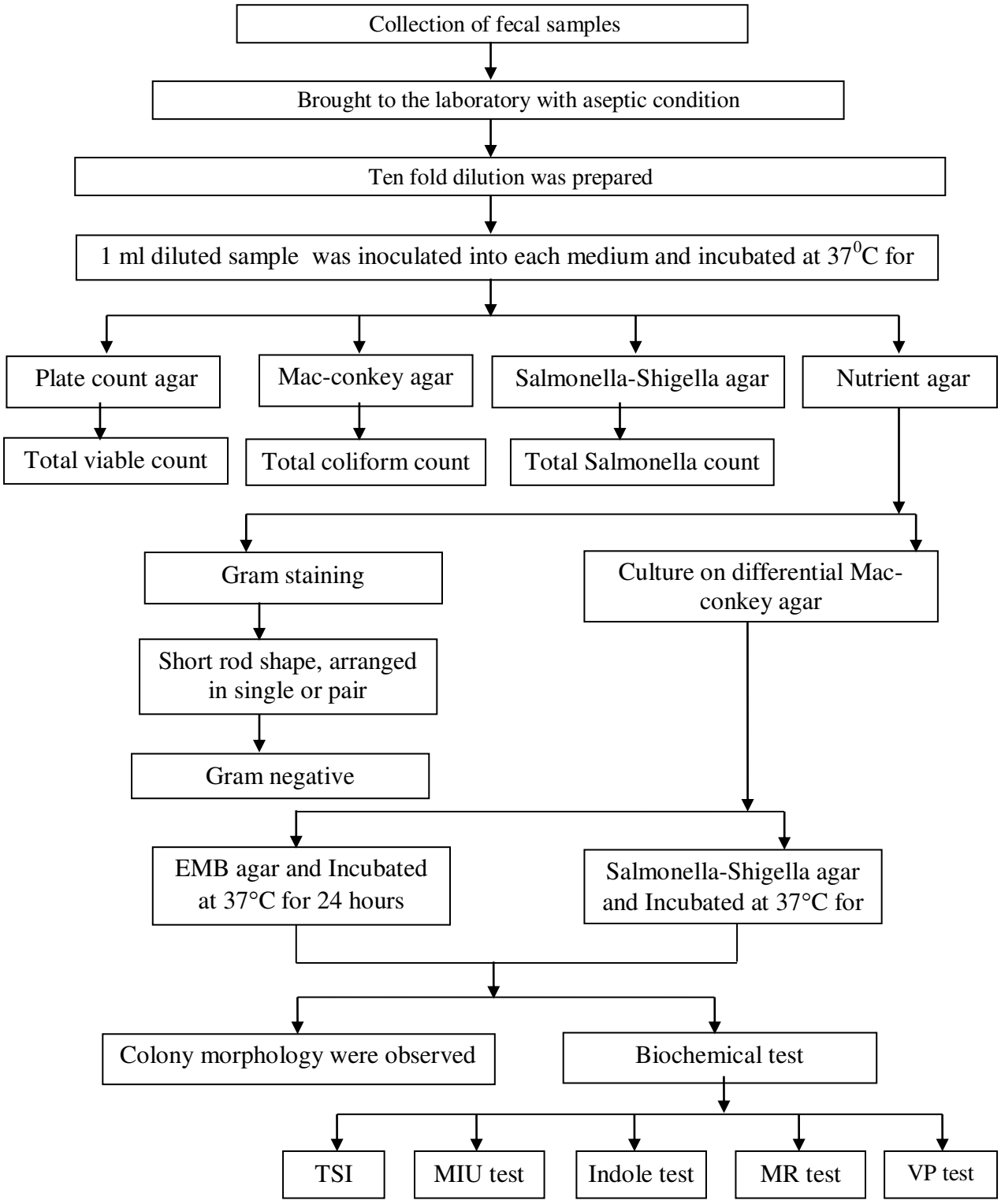


Fig. 1 The schematically illustration of layout of the experiment

3.2.2 Collection of fecal sample

Fecal samples from three different place of Dinajpur upazila were collected aseptically into small polybags which placed on ice box and brought to the bacteriology laboratory, Department of Microbiology, HSTU, Dinajpur with necessary precautions for bacteriological examination.

3.2.3 Sample preparation

1gm of each fecal sample mixed with 100 ml of 2% buffered peptone water by stirring done by stirring machine in a small conical flask.

3.2.4 Determination of bacterial load

3.2.4.1 Serial dilution of the sample

In serial dilution the original inoculum was diluted in a series of dilution tubes. At first 10 sterile test tubes were placed on a test tube holder rack containing 9 ml of 2% buffered peptone water. Then 1ml sample from 1^{st} test tube mixed with 2^{nd} test, then from 2^{nd} test tube to 3^{rd} test tube and finally 9^{th} to 10^{th} test tube and 1ml discard from 10^{th} test tube by the help of pippet and in every steps mixing was done properly.

3.2.4.2 Enumeration of total viable count (TVC)

For the determination of total bacterial count, 1 ml of each ten-fold dilution was transferred and spread on duplicate plate count agar using a fresh pipette for each dilution. The diluted samples were spread as quickly as possible on the surface of the plate with a sterile glass spreader. One sterile spreader was used for each plate. The plates were then kept in an incubator at 37^0 C for 24-48 hours. Following incubation, plates exhibiting 30-300 colonies were counted. The average number of colonies in a particular dilution was multiplied by the dilution factor to obtain the total viable count. The total viable count was calculated according to ISO (1995). The result of the total bacterial count was expressed as the number of organism or colony forming unit per ml (CFU/ml) of sample and multiplied with 100 to calculate the colony in 1gm of feces.

3.2.4.3 Enumeration of total *E. coli* count (TEC)

For the determination of TEC count 1ml of each tenfold dilution was transferred to EMB agar. For each dilution five test plates containing EMB agar were used. All the agar

plates were incubated at 37 ^{o}c temperature for 48 hours. The total *E. coli* count was calculated according to ISO (1995). The result of the TEC was expressed as the number of organism or colony forming units per gm (CFU/g) of sample.

3.2.4.4 Enumeration of total *Salmonella* Count (TSC)

For the determination of TSC, 1ml of each tenfold dilution was transferred to Salmonella-Shigella agar. For each dilution five test plate containing Salmonella-Shigella agar were used. All the agar plates were incubated at 37 ^{o}C temperature for 48 hours. The total salmonella count was calculated according to ISO (1995). The results of the total salmonella count was expressed as the number of organism or colony forming units per ml (CFU/ml) of sample and multiplied with 100 to calculate the colony in 1gm feces.

3.2.5 Isolation and identification of pathogens

The entire samples were selected for bacteriological culture.

3.2.5.1 Isolation and identification

3.2.5.1.1 Culture on ordinary media

Each fecal sample earlier put into transport media was divided and inoculated separately in nutrient agar (NA) and plate count agar to promote growth of bacteria. Each group of these media was incubated at 37^{0}C for overnight. The colonies on primary cultures were repeatedly sub-cultured by streak plate method (Cheesbrough, 1985)

3.2.5.1.2 Morphological characterization of organisms's Gram's staining method

- A loopful of sterile distilled water was placed in the center of a clean sterile slide.
- A small colony was picked up with a bacteriological loop and was mixed with distilled water on the slide.
- The colony was made to thin smear on a slide.
- The smears were fixed by air drying.
- 0.5% crystal violet solution was then applied on the smear for one minute.
- After washing Gram's iodine was then added to act as mordant for one minute and then wash again.
- Acetone alcohol was then added to decolorize for 1-2 seconds.

- Then the slide was washed with water.
- Safranine was added as counter stain and allowed for one minute.
- The slide was then washed with water.
- Then the slide was blotted with blot paper and was allowed to air dry. The slide was examined under microscope with high power objective (100X) using immersion oil.

3.2.5.2 Techniques used for the isolation and identification of *Escherichia coli.*

3.2.5.2.1 Culture into different media

Sterilized platinum loop was used for streaking the lactose broth culture on MacConkey and EMB agar to get isolates in pure culture. All inoculated media were kept at 37°C for overnight in an incubator.

3.2.5.2.1.1 MacConkey agar

Materials from lactose fermentation tubes were inoculated into MacConkey agar plates and were incubated.

3.2.5.2.1.2 Eosin Methylene Blue (EMB) agar

Materials from lactose fermentation tubes were inoculated into EMB agar plates which after incubation, showed metallic sheen if positive for *Escherichia coli.*

3.2.5.2.2 Microscopic study for identification of *Escherichia coli* suspected colonies by Gram's staining

Gram's staining was performed to determine the size, shape, and arrangement of bacteria. Gram staining reaction was performed according to the methods described by Merchant and Packer (1967).

3.2.5.2.3 Identification of *Escherichia coli* isolates by biochemical test

3.2.5.2.3.1 Indole test

Two ml of peptone water was inoculated with 5 ml of bacterial culture and incubated for 48 hours. Kovac's reagent (0.5 ml) was added and mixed thoroughly. The tube was then allowed to stand for a while.

3.2.5.2.3.2 Triple sugar iron (TSI) agar slant

The test organisms were culture into TSI agar slant by stab streak method.

3.2.5.2.3.3 Motility, Indole, Urease (MIU) test

Suspected colony was inoculated into the tube containing MIU medium. Then the medium was incubated at 37°C for overnight.

3.2.5.2.3.4 Methyl red test

The test was conducted by inoculating a colony of the test organism in 0.5 ml sterile glucose phosphate peptone broth. After 48 hours incubation at 37°C, a drop of methyl red solution was added. A red coloration is positive and indicates an acid pH of 4.5 or less resulting from the fermentation of glucose (Cheesbrough, 1984).

3.2.5.2.3.5 Voges-Proskaure test

2 ml of sterile glucose phosphate peptone water were inoculated with the 5ml of test organisms. It was incubated at 37°C aerobically for 48 hours. A very small amount (knifepoint) of creative was added and mixed 3 ml of α-naphthol were added and snaked well. The bottle cap was removed and left for an hour at room temperature.

3.2.5.3 Techniques for isolation and identification of *Salmonella* spp.

3.2.5.3.1 Culture into different media

Loopful aliquot was taken from the nutrient broth culture and streaked on nutrient agar media to get in pure culture. All inoculated media were kept at 37^0C for overnight in an incubator.

3.2.5.3.1.1 Nutrient agar

Materials from nutrient broth tubes were inoculated into nutrient agar plates.

3.2.5.3.2 Microscopic study for identification of *Salmonella* spp. suspected colonies by Gram's staining

Gram's staining was performed to determine the size, shape, and arrangement of bacteria. Gram's staining reaction was performed according to the methods described by

Merchant and Packer (1967). Stained slides were examined under light microscope at 100 x magnification.

3.2.5.3.3 Identification of *Salmonella* spp. isolates by biochemical test

3.2.5.3.3.1 Triple sugar iron (TSI) agar slant

The test organisms were culture into TSI agar slant by stab streak method and incubated at 37^0 C.

3.2.5.3.3.2 Indole test

Two ml of peptone water was inoculated with 5 ml of bacterial culture and incubated for 48 hours. Kovac's reagent (0.5 ml) was added and mixed thoroughly. The tube was then allowed to stand for a while.

3.2.5.3.3.3 Methyl red test

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3.2.5.3.3.4 Voges-Proskaure test

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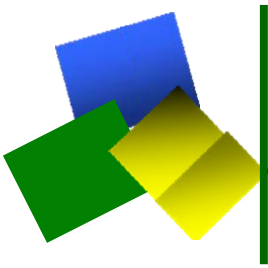


CHAPTER IV

RESULTS

CHAPTER IV

RESULTS

4.1 Bacterial load

4.1.1 Enumeration of total viable count (TVC)

The result in table 1 reveals that average total viable bacterial count (TVC/gm of fecal sample) of rabbit of group A, group B and group C were 1.2×10^4, 2.4×10^6, 3.5×10^7 CFU/gm of sample respectively.

Table 1. Density of average total viable count

Sl. No.	Group	No. of animal analyzed	Average Total viable count CFU/g sample	Average Total viable count log10/g
1	A	10	1.2×10^4	4.08
2	B	10	2.4×10^6	6.38
3	C	10	3.5×10^7	7.54

4.1.2 Enumeration of total *E. coli* count (TEC)

The result in table 2 reveals that average total *E. coli* count (TEC/gm of fecal sample) of rabbits of group A, group B and group C were 2.5×10^3, 5.5×10^5 and 4.5×10^6 CFU/gm of sample respectively.

Table 2. Average total *E. coli* count

Sl. No.	Group	No. of animal analyzed	Average Total *E.coli* CFU/g sample	Average Total *E. coli* count log10/g
1	A	10	2.5×10^3	3.40
2	B	10	5.5×10^5	5.74
3	C	10	4.5×10^6	6.65

4.1.3 Enumeration of total *Salmonella –shigella* count (TSSC)

The result in table 3 reveals that average total Salmonella spp. Count (TSC/gm of fecal sample) of rabbits of group A, group B and group C were 1×10^2, 2.1×10^2 and 3×10^3, respectively.

Table 3. Average total *Salmonella-shigella* count

Sl. No.	Group	No. of animal analyzed	Average Total *Salmonella spp* CFU/g sample	Average Total *salmonella* count log10/g
1	A	10	1×10^2	2.00
2	B	10	2.1×10^2	2.32
3	C	10	3×10^3	3.47

4.2 Identification of *Escherichia coli* by different bacteriological methods

4.2.1 Results of cultural examination

4.2.1.1 MacConkey (MC) agar

On Macconkey agar bright pink colord organism produced (Plate 10).

4.2.1.2 Eosin methylene blue (EMB) agar

EMB agar plates streaked separately with the organism revealed the growth of bacteria after 24 hours of incubation at 37^0C aerobically. The growth was indicated by smooth, circular, black color colonies with metallic sheen on the agar plate (Plate 8).

4.2.2 Results of Gram's staining

The microscopic examination of Gram's stained smears from Macconkey and EMB agar revealed Gram-negative, pink colored, small rod shaped organisms arranged in single, pairs or short chain (Plate 9).

4.2.3 Results of biochemical test

4.2.3.1 Triple sugar iron (TSI) agar slant test

Triple sugar iron (TSI) agar slant reaction showed yellow color butt, gas positive, no H_2S production by *E. coli* (Plate 18).

4.2.3.2 Motility, Indole, Urease (MIU) test

Escherichia coli in MIU medium caused turbidity and urease production with indole positive (Plate 14).

4.2.3.3 Other biochemical tests

All the isolates were indole test positive, methyl-red positive and VP test negative. Results of these tests are shown in Table 4 and Plate 15, 16, and 17.

Table 4. Result of biochemical test for *E. coli*

Different biochemical test	Result
Triple sugar iron (TSI) agar slant test	slant reaction showed yellow color butt, gas positive, no H_2S production
Motility, Indole, Urease (MIU) test	turbidity and urease production with indole positive
Indole	Bright pink color ring
MR	Red color
VP	No color change

4.3 Identification of *Salmonella* spp. by different bacteriological methods

4.3.1 Results of cultural examination

4.3.1.1 *Salmonella-shigella* agar

On *Salmonella-shigella* agar black centered, smooth small round colony produced (Plate 11).

4.3.1.2 Brilliant Green Agar

On Brilliant Green Agar organisms produces pale pink colour colonies against a pinkish background (Plate 12).

4.3.2 Results of Gram's staining

The organisms were observed as Gram-negative small rod shaped organism arranged singly or pairs (Plate 13).

4.3.3 Results of biochemical test

4.3.3.1 Triple sugar iron (TSI) agar slant test

In TSI agar slant, red slant, yellow butt, gas positive, and presence of black precipitate indicate H_2S positive (Plate 19).

4.3.3.2 Other biochemical tests

All the isolates were methyl-red positive, Voges-Proskauer test negative, Motility, Indole, Urease (MIU) test and indole test negative. Results of these tests are shown in Table 5.

Table 5. Result of biochemical test for *Salmonella spp*

Different biochemical test	Result
Triple sugar iron (TSI) agar slant test	yellow butt, red slant and black colour in the slant due to hydrogen sulfied production
Motility, Indole, Urease (MIU) test	Positive.
Indole	Negative
MR	Red color
VP	No color change

Table 6: Cultural characteristics of organisms of different cultural media.

Sl. No.	Media used	Colony characteristics	Organisms
1	Mac-conkey agar	Bright pink colored colonies	*E. coli*
2	EMB agar	Smooth, circular, black color colonies with metallic sheen	*E. coli*
3	*Salmonella-shigella* agar	Black centered, smooth, small round colony	*Salmonella spp.*
4	Brilliant green agar	Pale pink colour colonies agaist a pinkish background	*Salmonella spp.*

4.4 Prevalence of *E. coli* bacteria

Total 30 samples were tested where *E. coli* found in 13 sample prevalence was 43.33%, where prevalence of *E. coli* on group A was 30%, Group B was 40% and group was C 60% (Table 7).

Table 7. Prevalence of *E. coli* bacteria

Sl. No.	Group	Age (months)	Management system	Total no. of sample analyzed	No. of positive for *E. coli*	Prevalence (%)
1	A	4	intensive	10	3	30
2	B	4	intensive	10	4	40
3	C	4	intensive	10	6	60
Total				30	13	43.33

4.5 Prevalence of *Salmonella spp.*

Total 30 samples were tested and 7 samples showed positive characteristics for *Salmonella spp,* prevalence was 23.33% where prevalence on group A was 10%, Group B was 20% and group C was 40% (Table 8).

Table 8. Prevalence of *Salmonella spp.*

Sl. No.	Group	Age (month)	Management system	Total no. of sample analyzed	No. of positive for *salmonella spp.*	Prevalence (%)
1	A	4	intensive	10	1	10
2	B	4	intensive	10	2	20
3	C	4	intensive	10	4	40
Total				30	7	23.33

4.6 Effects bacteria on the basis of growth performance (body weight)

All rabbit were same aged, but had different body weight and had different bacterial load on feces. The body weight of group A, group B and group C were 1.5, 1.2, 1 kg respectively. Where group C had highest bacterial load (7.54 Log 10/g) and group A had lowest bacterial load (4.08Log10/g) (Table 9).

Table 9. Effects bacteria on the basis of growth performance (body weight)

Sl. No.	Group	Body weight(kg)	Total value count Log10/g	Total *E. coli* count Log10/g	Total *Salmonella* count Log10/g
1	A	1.5	4.08	3.40	2.00
2	B	1.2	6.38	5.74	2.32
3	C	1	7.54	6.65	3.47

Plate 1: Fecal sample

Plate 2: Diluted fecal sample.

Plate 3: Ten fold dilution of fecal sample.

Plate 4: Colony count in counter machine.

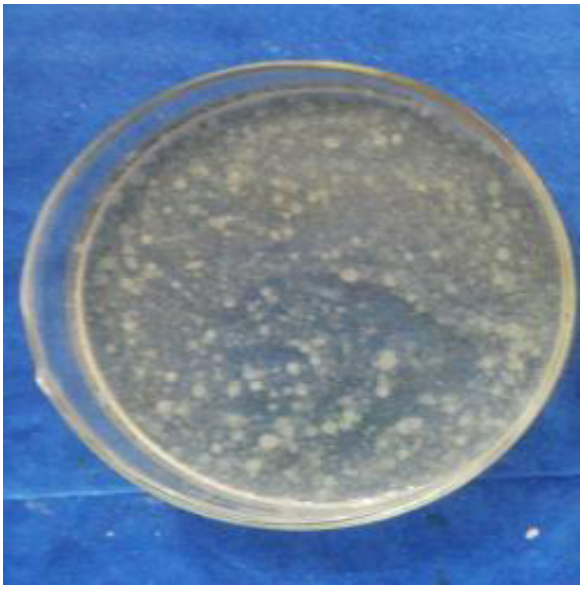


Plate 5: Colony of bacteria in plate count agar for TVC

Plate 6: Colony of bacteria in MacConkey agar

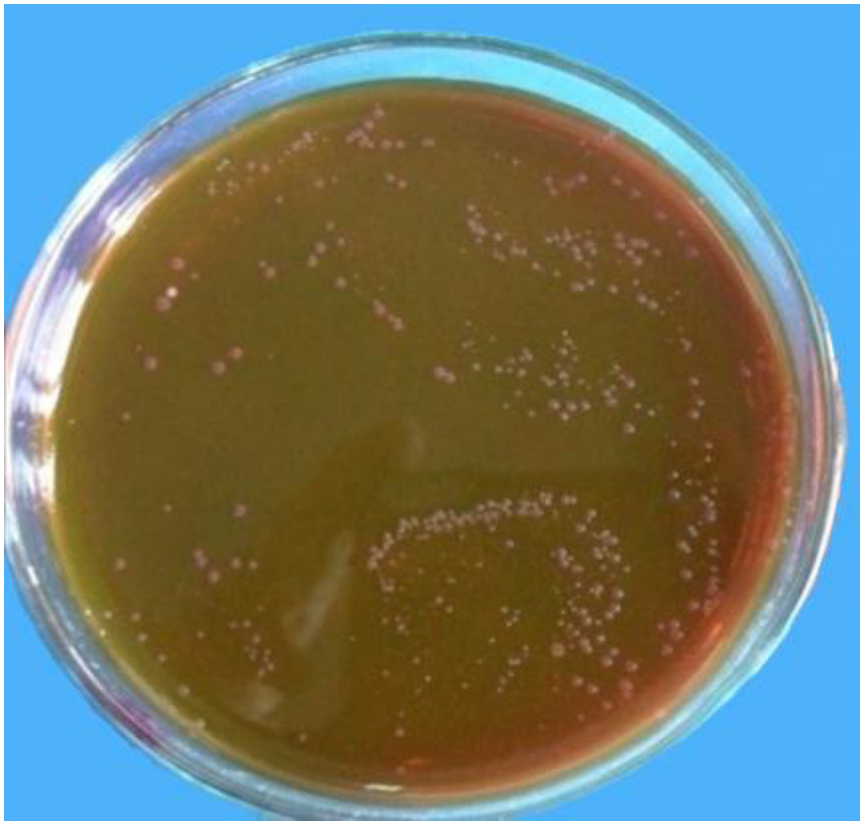


Plate 7: Colony of bacteria in SS agar

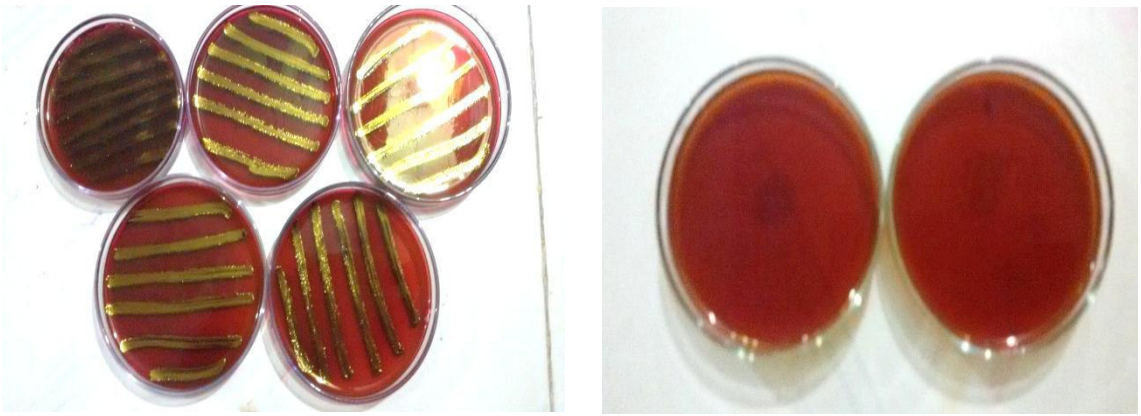


Plate 8: Metallic sheen produced by *E. coli* on EMB agar (left) and control (right).

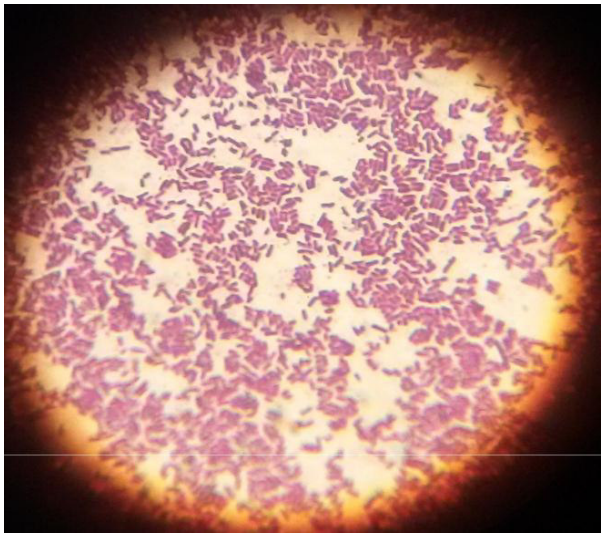


Plate 9: *E. coli* showing pink colour small rod-shape, arranged in single or pair at 100x magnification (Gram's staining).

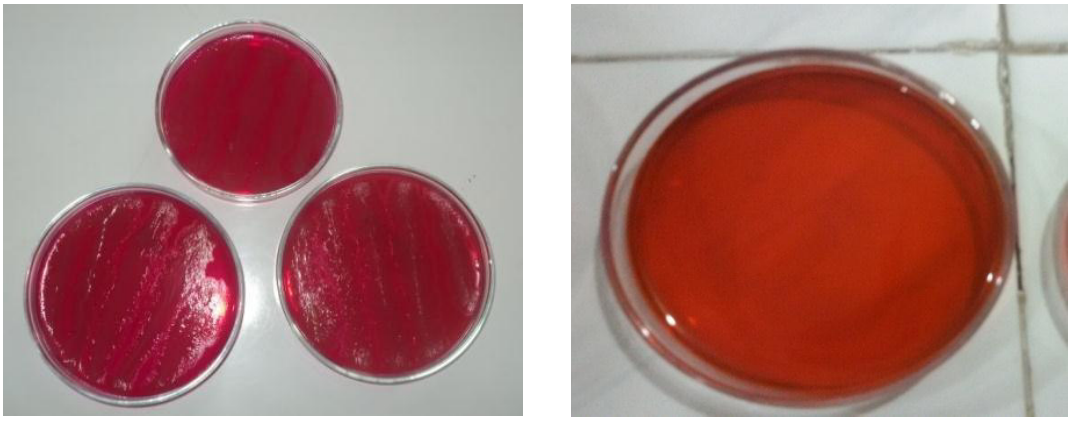


Plate 10: *E. coli* produced bright pink colored colonies on MacConkey agar (left) and control (right).

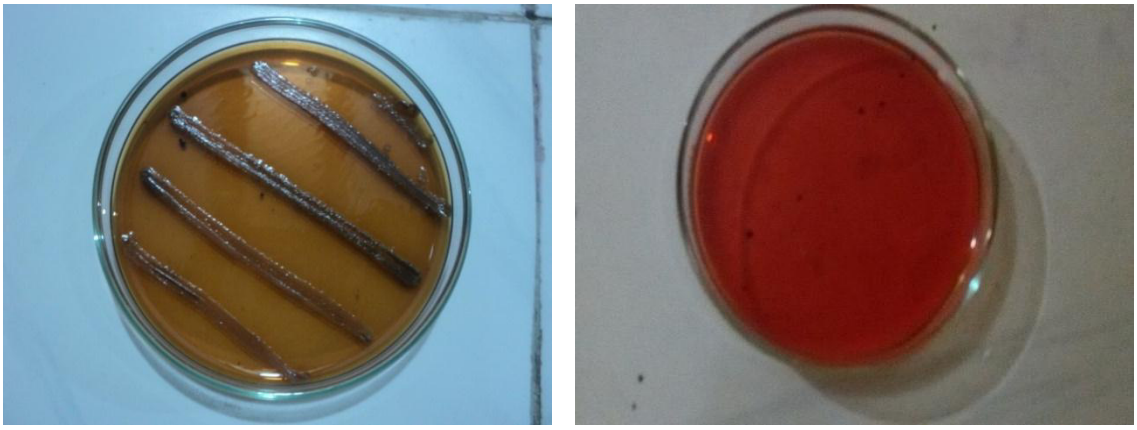


Plate 11: Growth of microorganism in Salmonella-Shigela (SS) agar as black centered, smooth, small round colony (left) which compare with control petridis (right)

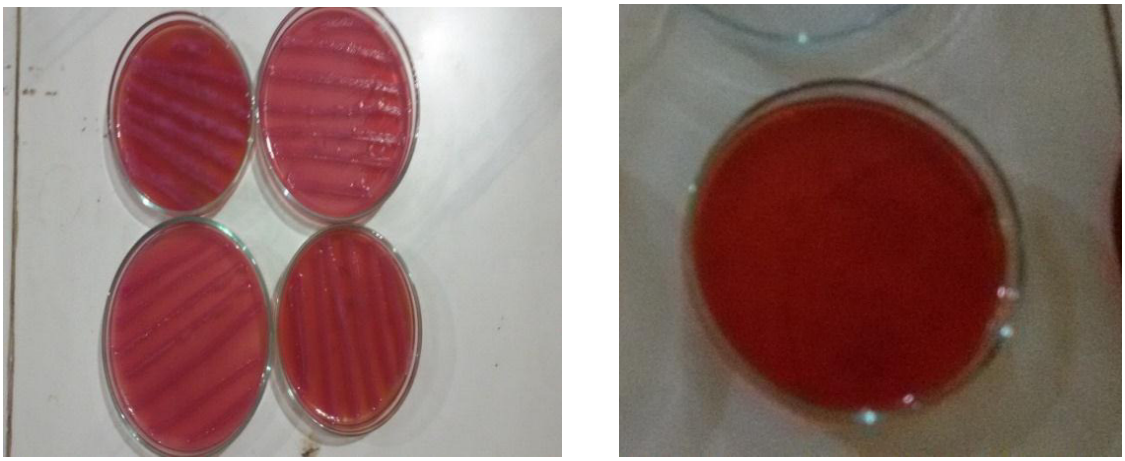


Plate 12: Growth of *Salmonella spp* in Brilliant Green Agar showing pale pink colour colonies agaist a pinkish background (left) and control (right)

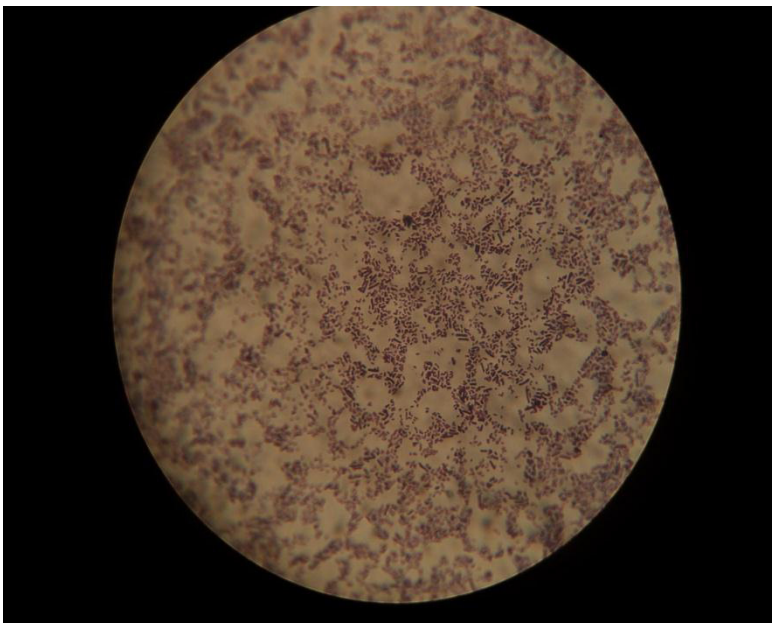


Plate 13: *Salmonella spp* showing pink colour, small rod-shape, arranged in single or pair at 100x magnification (Gram's staining).

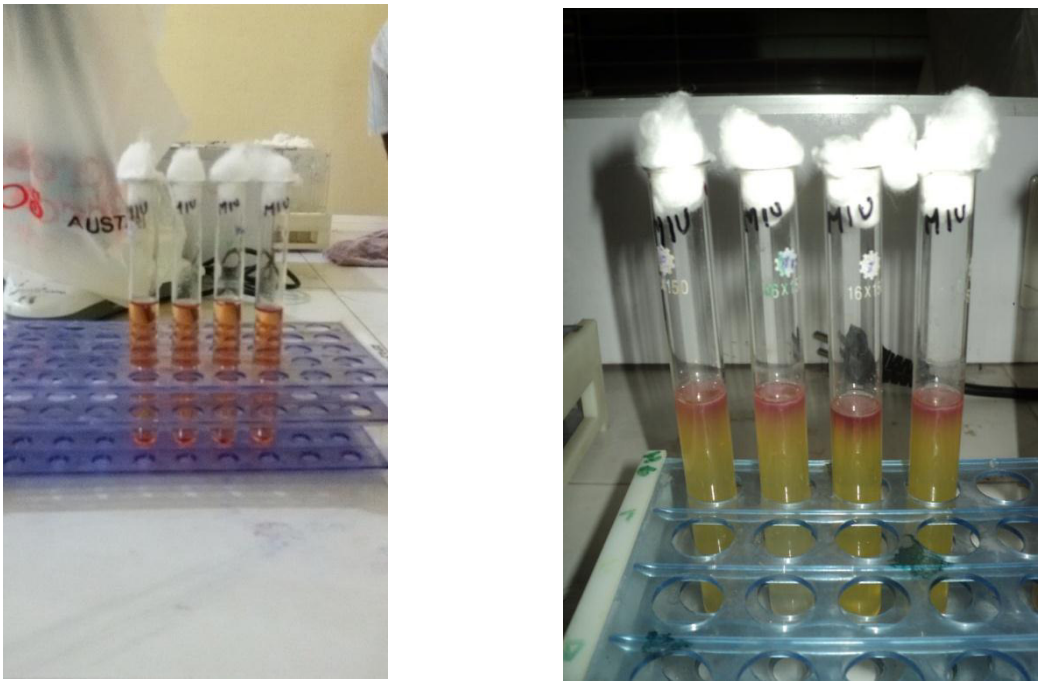


Plate 14: Motility Indole Urease (MIU) test causing turbidity and urease production with indole positive by *E. coli* (right) and control (left)

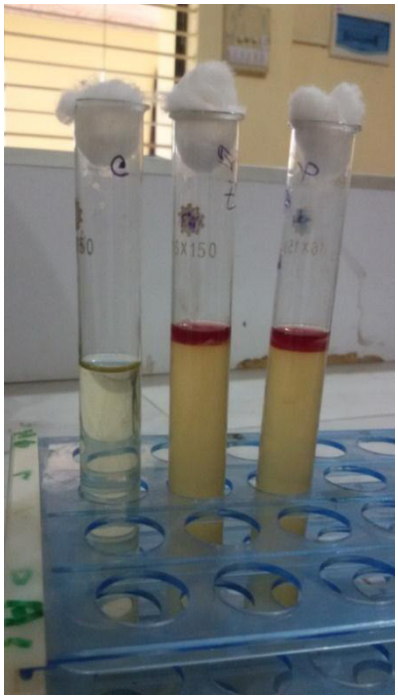


Plate 15: Indole test showing positive results with a red color in the reagent layer indicating indole production with reaction of *E. coli* (right) and control (left).

Plate 16: Methyl-Red test for *E. coli* showing the medium was changed to bright red color (right) and control (left).

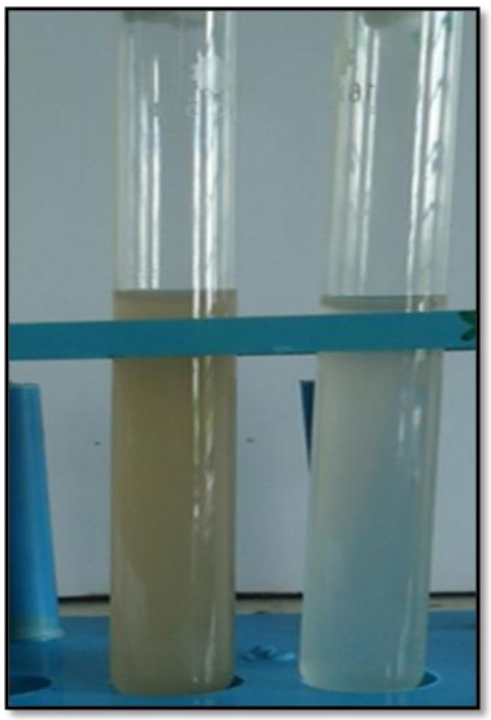


Plate 17: Voges-Proskauer test for *E. coli* showing no change of the medium (left) and control (right)

Plate 18: Culture on triple sugar iron (TSI) agar slant reaction showing yellow color butt and slant (left) indicate,gas produce but no H_2S production by *E. coli* and control (right)

Plate 19: Triple Sugar Iron (TSI) slant test showing yellow butt, red slant and black colour in the slant due to hydrogen sulfied production (right), and control (left)

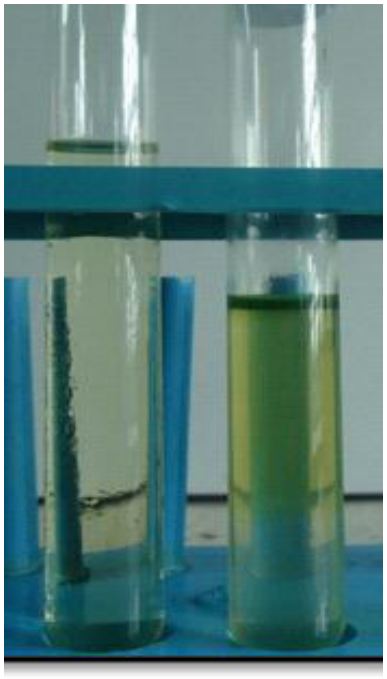


Plate 20: Indole test showing no change of the medium with the reaction of *Salmonella spp* (left) and control (right).

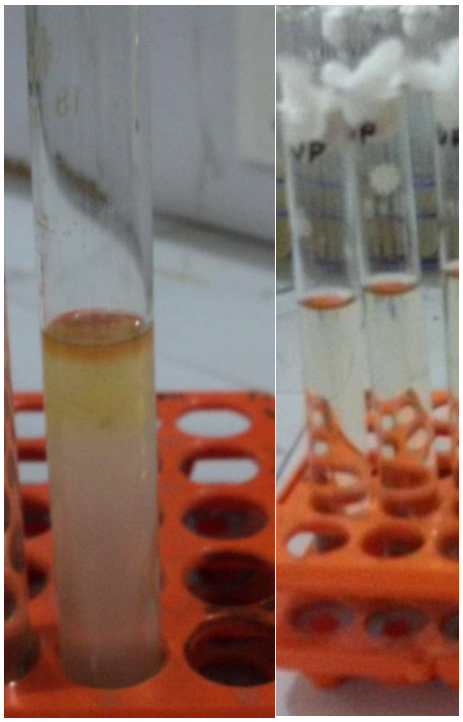
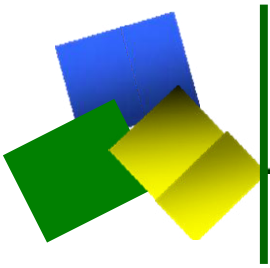


Plate 21: *Salmonella spp* show no colour change on indole test (left), compared with control (right)



CHAPTER V

DISCUSSION

CHAPTER V

DISCUSSION

The present study was undertaken to determine the effect of bacterial load on body growth performance of rabbit. 30 rabbits (4 months) were selected randomly from Dinajpur upazila Dinajpur. All the rabbits were reared in intensive condition. Then fecal sample was collected and brought to the microbiology department of HSTU. These rabbit were divided into three groups according to their body weight gain and feeding management.

The rabbits of group A (Average body weight 1.5 kg) were fed napier grass, pellets and group B (Average body weight 1.2 kg) were fed napier grass, vegetables and group C (Average body weight 1 kg) were fed only of vegetables.

The present study showed that among 30 tested samples, The TVC/g fecal sample found on group A, group B and group C were 1.2×10^4, 2.4×10^6, 3.5×10^7 CFU/gm of sample. *E. coli* load on three different samples of 3 groups of rabbit were not uniform but varied quite considerably. The *E. coli* counts/g sample on group A, group B and group C were 2.5×10^3, 5.5×10^5 and 4.5×10^6 CFU/g of sample and *Salmonella spp.* load were 1×10^2, 2.1×10^2 and $3x\ 10^3$, CFU/g sample on group A, group B and group C respectively. Group C had highest bacterial load 3.5×10^7 CFU/gm of sample (7.54 Log 10/g) and group A had lowest bacterial load 1.2×10^4 CFU/gm of sample (4.08 Log10/g).

Highest total viable count found 3.5×10^7 CFU/gm feces was found in rabbits of 1kg body weight, and lowest count $1.2x\ 10^4$ CFU/gm feces was found in rabbit of 1.5kg body weight. Where highest *E. coli* count was 4.5×10^6 CFU/gm of feces of a rabbit body weight 1 kg and lowest count was 2.5×10^3 CFU/gm of feces of a rabbit with body weight 1.5 kg. Where highest *Salmonella spp* colony count 3×10^3 CFU/gm of feces of rabbit, body weight1 kg and lowest was 1×10^2 CFU /gm of feces of rabbit whose body weight 1.5 kg.

From total 30 samples, *E. coli* found in 13 sample, was 43.33%, where percentage of *E. coli* on group A was 30%, Group B was 40% and group C was 60%.

From total 30 samples, 7 samples showed positive characteristics for *Salmonella spp,* percentage was 23.33% where percentage on group A was 10%, Group B was 20% and group C was 40%.

Highest *E. coli* 4. 5×10^6 CFU/gm feces and salmonella count 3×10^3 CFU/gm of feces were found in rabbits of group c which body weight was 1 kg. Lowest *E. coli* 2. 5×10^3 CFU/gm feces and salmonella count 1x 102CFU/gm of feces were found in rabbits of group c which body weight was 1.5 kg.

It can be said that higher bacterial load lower body weight and lower bacterial load higher body weight. From this study it was evident that those rabbits fed only of vegetables caused diarrhea and over growth of opportunistic bacteria as a result their body weight were decreased which was supported by Lea and Febinger (1995).

Characterization results of the study indicated that the contaminated by bacteria. A clear finding of the colony characteristics of the isolates was observed. The feces isolates were able to produce pale red colour colony on BGA agar, characteristic metallic sheen colony on EMB agar and bright pink colored colony on MacConkey agar.

In Gram's staining, the organisms revealed gram negative, pink colored with rod shaped appearance and arranged in single or in pair were suspected as *E. coli* which supported by Zinnah *et al.* (2007) and *Salmonella spp* exhibit Gram negative, small rod, single or paired in arrangement under microscope which was supported by the other researchers (Cheesbrough, 1985).

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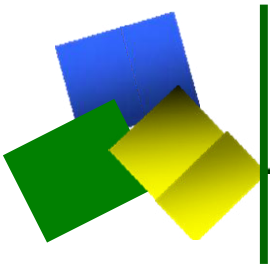
In the present study, biochemical tests which were used for characterization of bacterial pathogens also used by Kara *et al.* (2004). *E. coli* gave positive reaction to indole, Motility Indole Urease, Methyl red and salmonella isolates from rabbit feces was indole negative, not produced red color, absence of turbidity on MIU test indicated non-motile organism, on TSI agar slant produce yellow butt and slunt, MR test positive but VP test negative which supported by American Type Culture Collection.



CHAPTER VI

CONCLUSIONS

CHAPTER VI

CONCLUSIONS

The present study was conducted for determination of bacterial loads on rabbit feces and growth performance of rabbit. A total of 30 fecal samples of rabbit collected randomly from Dinajpur District of Bangladesh. Rabbit are of different body weight, a series of tests were performed for the isolation, identification and total viable count of different types of bacteria and digital weight balanced (Mega digital scale) were used to know the body weight, birth weight of rabbit. Different types of ordinary, enriched and selective media such as nutrient agar, Plate count agar, MacConkey agar, Eosin methylene blue agar, Triple sugar iron agar, Brilliant green agar, Nutrient agar and Triple Sugar Iron agar slant were used for the determination of the cultural characteristics of the different types of isolates. Biochemical properties of the isolated bacteria were studied by MR, VP and indole tests.

From the present study, it was concluded that rabbit having higher microbial load have lower body weight.

In the context of this study, it may be concluded that,

- Total viable count of organisms was successfully performed from different fecal samples.
- *E. coli* and *Salmonella spp.* were present and could be isolated from fecal samples.
- *E. coli* and *Salmonella spp.* were successfully isolated and confirmed by different bacteriological media and biochemical reaction.
- Body weight was measured accurately.
- Hygienic measure should be taken to ensure that the collection, handling, processing, distribution and storage of sample.

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APPENDICES

APPENDICES

APPENDIX 1

Composition of Different Media

1. Nutrient agar (Hi Media)

Ingredients:	**g/L**
Peptic digest of animal tissue	5.0
Sodium chloride	5.0
Beef extract	1.5
Yeast extract	1.5
Final pH (at 250C)	7.4 ± 0.2

2. Eosine methylene blue Agar (Hi Media)

Ingredients:	**g/L**
Peptic digest of animal tissue	10
Lactose	5.0
Sucrose	5.0
Dipotassium phosphate	2.0
Eosin - Y	0.40
Methylene blue	0.065
Agar	20.0
Final pH (at 250C)	7.2 ± 0.2

3. MacConkey agar (Hi Media)

Ingredients:	**g/L**
Peptic digest of animal tissue	17.0
Protease peptone	3.0
Lactose monohydrate	10
Bile salt	1.5
Sodium chloride	5.0
Agar-agar	15.0
Neutral red	0.03
Final pH (at 250C)	7.1 ± 0.2

4. TSI agar (Hi Media)

Ingredients:	**g/L**
Peptic digest of animal tissue	10.00
Casein enzymic hydrolysate	10.00
Yeast extract	3.00
Beef extract	3.00
Lactose	10.00
Sucrose	10.00
Dextrose	1.00
Sodium chloride	5.00
Ferrous sulphate	0.20
Sodium thiosulphate	0.30
Phenol red	0.024
Agar	12.00
Final pH(at 25°C)	7.4 ± 0.2

5. MIU medium base (Hi Media)

Ingredients:	**g/L**
Casein enzymic hydrolysate	10.00
Dextrose	1.00
Sodium chloride	5.00
Phenol Red	0.01
Agar	2.00
Final pH(at 25°C)	6.8 ± 0.2

6. MR-VP medium (Hi Media)

Ingredients:	**g/L**
Buffered peptone	7.00
Dextrose	5.00
Dipotassium phosphate	5.00
Final pH (at 25°C)	6.9 ± 0.2

7. Peptone water

Ingredients:	**g/L**
Peptone	1.00 gm
Distilled water	1000 ml

APPENDIX 2

Preparation of reagents

1. Kovacs reagent

P-dimethyl aminobenzal dehyde	5 gm
Amylalcoho	l75 gm
Conc. HCL	25 ml

2. V-P reagent 1

5% alpha –naptholin absolute ethyl alcohol

3. V-P reagent 2

40%potassium hydroxide containing 0.3 creatine. The ingredients were dissolved by heating gently over steam bath. When in solution add 0.05gm of cotton blue dye.

4. Phosphate buffered solution

Sodium chloride	8 gm
Disodium hydrogen phosphate	2.8 gm
Potassium chloride	0.2 gm
Potassium hydrogen phosphate	0.2 gm
Distilled water to make	1000 ml

5. Methyl red solution

Methyl red	0.05 gm
Ethanol (absolute)	28 ml
Distilled water	22 ml

6. Phenol red solution

0.2% aqueous solution of phenol red

7. Potassium hydroxide solution

40% aqueous solution of KOH

8. Gram stain solution

 Stock crystal violet

Crystal violet	10 gm
Ethyl alcohol (95%)	1000 ml

 Stock oxalate solution

Ammonium oxalate	1 gm
Distilled water	1000 ml

 Lugols iodine solution

Iodine crystal	1 gm
Potassium iodide	2 gm

 Ethyl alcohol 250 ml

 Acetone 250 ml

 Counterstain

Safranine	2.5 ml
Ethyl alcohol (95%)	100 ml

Safranine working solution

The stock safranine is diluted 1:4 with distilled water.