Risk factors associated with diarrhoeal disease and diarrheagenic *E.coli* disease in Duc Giang Hospital, north-eastern of Ha Noi, Viet Nam

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Table content

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List of Acronyms

AA	:	aggregative adherence
A/E	:	attaching and effacing
bfp	:	Bundle forming pilus
bp	:	Base pair
DEC	:	Diarrheagenic *Escherichia coli*
DNA	:	Deoxyribonucleic acid
E. coli	:	*Escherichia coli*
eae	:	*E.coli* attaching and effacing gene
EAEC	:	Enteroaggregative *Escherichia coli*
EHEC	:	Enterohaemorrhagic *Escherichia coli*
EIEC	:	Enteroinvasive *Escherichia coli*
EPEC	:	Enteropathogenic *Escherichia coli*
ETEC	:	Enterotoxigenic *Escherichia coli*
ELISA	:	Enzyme linked immunosorbent assay
HUS	:	Haemorrhagic uremic syndrome
LT	:	Labile toxin
NIHE	:	National Institute of Hygiene and Epidemiology, Ha Noi Viet Nam
ORT	:	Oral rehydration therapy
OR	:	Odds ratio
PBS	:	Phosphate buffered saline
P/F	:	P value and fisher exact test
PCR	:	Polymerase chain reaction
SMAC	:	Sorbitol-MacConkey (medium)
SPSS	:	Statistical Package for Social Sciences
ST	:	Heat stable toxin
stx	:	Shiga toxin gene
STEC	:	Shiga toxin-producing *E. coli*
VT	:	Verotoxin/Verocytotoxin
VTEC	:	Verocytotoxin-producing *Escherichia coli*
WHO	:	World Health Organisation

Summary

Risk factors associated with diarrhoeal disease and diarrheagenic *E. coli* disease in Duc Giang Hospital, north-eastern corner of Ha Noi, Viet Nam

Background:

Diarrheagenic *E. coli* (DEC) disease is more prevalent in infants and children is a very important cause of sporadic diarrhoea cases and diarrhoea outbreaks in many countries. Currently, DEC also stands as a chief causal factor for diarrhoea among travellers.

DEC disease is usually transmitted through food or water contaminated with human or animal faeces. Person-to-person transmission might also take place, but is probably less common. Poor sanitation, personal hygiene and environmental conditions are some of the factors that facilitate the transmission of the disease. Thus, DEC disease is more prevalent in developing countries.

Viet Nam is a tropical country in Southeast Asian area with low social-economic level. It has a high prevalence of diarrhoea. According to a report from Ministry of Health, there were over 1.000.000 hospital cases of diarrhoea that occurred in Viet Nam in 2002. Recent literature has shown that *E. coli* is the most important etiologic agent that causes diarrhoea in Viet Nam (2).

To date, there has not been any study about risk factors associated with DEC disease in these areas and with a focus on children less than 5 in Ha Noi. Such studies could contribute to reduce the morbidity and mortality from the DEC diseases and prevent outbreaks caused by DEC.

Objectives: The objectives of this study was to identify the risk factors associated with diarrhoeal disease and DEC disease in Long Bien and Gia Lam Districts and to identify diarrhoeagenic *E. coli* in diarrhoea patients in Duc Giang Hospital.

Methods:

A hospital-based case-control study was performed. DEC cases were defined by positive stool cultures and/or PCR test from suspected cases who were admitted to hospitals between July and December 2005. Controls were randomly chosen among patients in Duc Giang Hospital and were matched by sex and age. Interviews were performed using a standard questionnaire collecting information regarding their recent contacts with diarrhoeal disease, patient's history of travel, eating habits, hygiene and socio-economic indicators.

Results:

Between July and December 2005, 62 DEC cases that were recorded and 124 diarrhoeal controls and 62 non-diarrhoeal controls were selected. 79% of cases were among children under 5 years of age.

In a conditional logistic regression model for analyzing diarrhoeal versus non-diarrhoeal group, **negligence of washing hands before eating** (OR = 9.7, 95% CI 3.9 – 24.0, P = <0.001); **keeping food outside the fridge** (OR = 3.6, 95% CI 1.9 – 6.5, P = <0.001); were independently associated with diarrhoeal disease.

In a conditional logistic regression model for analyzing the DEC group versus the non-diarrhoeal group, **having contact with diarrhoeal patients** (OR = 4.5, 95% CI 1.3 – 16.0, P = 0.02); **negligence of washing hands before eating** (OR = 9.2, 95% CI 4.1 – 21.0, P = <0.001); **keeping food outside the fridge** (OR = 4.1, 95% CI 1.9 – 8.7, P = <0.001) were independently associated with DEC disease.

In a conditional logistic regression model for analyzing the DEC group versus the non-DEC diarrhoeal group, **eating outside more than 1-2 times/month** (OR = 4.2, 95% CI 1.8 – 9.6, P = <0.001); **roasted meat eating last week** (OR = 7.7, 95% CI 2.3 – 25.7, P = 0.001); **drinking pond water** (OR = 4.8, 95% CI 1.4 – 16.6, P = 0.013) were independently associated with DEC disease

Conclusion:

Our study suggested that **unsafe drinking water**, **keeping food outside the fridge** and **negligence of washing hands before eating** were risk factors for both DEC disease and other diarrhoeal diseases. **Eating outside more than 1-2 times/month**, **having contact with diarrhoeal patients**, **eating roasted meat last week** were risk factors for DEC disease.

These findings suggest to the policy makers: safe water should be providing to the community; Regular quality control of foods and beverages sold in restaurants.

Hygienic practices should be promoted by providing community health education on the importance of washing hands before eating; boiling drinking water; storing food in refrigerators (for those who have it), warming up food before eating; hand and food hygiene after contact with a patient who has diarrhea

Key words: Diarrhoeagenic *E. coli*, risk factors, Vietnam, diarrhoea, and developing countries.

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Introduction

Diarrhoeal diseases, especially in children, is a major public health problem in developing countries (3;4). *Escherichia coli (E. coli)* is one of the most important agents that cause diarrhoeal diseases (5;6).

Among the *E. coli* causing intestinal diseases, there were 5 well-described categories (5): enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), and enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), enterohemorhagic *E. coli* (EHEC). Among them EPEC, ETEC, EIEC are more common in developing countries, while EHEC was more prevalent in developed countries (5)**.**

Diarrheagenic *E. coli* (DEC) disease is more prevalent in infants and children (5;7-10) and is a very important cause of sporadic diarrhoeal cases and diarrhoeal outbreaks in many countries (5;9). Currently, DEC also stands as a chief causal factor for the diarrhoea among travellers. (11-13)

DEC disease was usually transmitted through food or water contaminated with human or animal faeces. Person-to-person transmission might also take place, but is probably less common (5;14-20). Poor sanitation, personal hygiene and environmental conditions are some of the factors that facilitate the transmission of the disease. Thus, DEC disease is more prevalent in the developing countries. Viet Nam is a tropical country in Southeast Asia from with low social-economic level and high prevalence of diarrhoea. According to a reported from Ministry of Health, there are over 1.000.000 hospital cases of diarrhoea occurred in Viet Nam in 2002.

Ha Noi is the capital of Viet Nam in centre of Red River delta with high population density. According to a reported from National Institute of Hygiene and Epidemiology, there were 51.906 cases of diarrhoea in Ha Noi in 2004.

Long Bien and Gia Lam District is an area in the north-eastern corner of Ha Noi City beyond the Red River with a high population density. Most people live in the rural area. The income of most of these families is based on cultivation and breeding. It is the main city area to provide food such as beef, pork, vegetables, and fruits to Ha Noi city and other provinces. Recent literature has shown that *E. coli* is the most important etiologic agent that causes diarrhoea in Viet Nam (2).

To date, there was no study about risk factors associated with transmission DEC disease in these areas and with a focus on children less than 5 in Ha Noi.

Such studies could contribute to reduce the morbidity and mortality from the DEC diseases and prevent outbreaks caused by DEC. The proposed study was conducted in Duc Giang Hospital, north-eastern corner of Ha Noi, Viet Nam in 2005-2006 with the intended objectives.

Chapter I: Literature review

1.1. Burden of diarrhoeal diseases caused by Escherichia coli

E. coli accounts for an estimated 780-900 million cases of diarrhoea worldwide annually (21). Although most strains of *E. coli* are harmless, the pathogenic strains represent the most common cause of diarrhoea in the developing world (22).

Among seven different classes of pathogenic *E. coli*, ETEC, EPEC and EIEC are responsible for the vast majority of diarrhoeal cases in the developing world (23).

ETEC was an important cause of diarrhoea in infants and travellers in developing countries or regions with poor sanitation. Studies of children in these settings (2;5;8;9;24;25), have reported ETEC to be the most frequently isolated entero-pathogen, accounting for approximately 210 million diarrhoeal episodes and approximately 380,000 deaths annually (26). A study (8) conducted in Egypt found that the incidence of ETEC diarrhoea among Egyptian children was 1.5 episodes per child per year and accounted for 66% of all first episodes of diarrhoea after birth. The incidence increased from 1.7 episodes per child per year in the first 6 months of life to 2.3 in the second 6 months and declined thereafter.

EPEC is a common cause of diarrhoea among infants in developing countries (5;24). Disease due to EPEC is rare in developed countries. In poorer countries, where EPEC is more prevalent, it is found almost exclusively among infants; especially among those aging less than 6 months (5). According to a report that compiles data from 69 countries, EPEC causes about 240,000 annual deaths among children under five. Some types of EPEC were referred to as entero-adherent *E. coli* (EAEC), based on specific patterns of adherence. EPEC and EAEC were found to be the etiological agents for traveller's diarrhoea in the Asian countries (27). EAEC has been shown to be associated with persistent diarrhoea in adults with HIV in the United States and Switzerland (6;28).

EIEC is disease closely related to the *Shigella* species and might cause very similar (if not identical) to that caused by *Shigella*. Diseases caused by the EIEC appear to be rather uncommon. However, this was owing to the fact that diarrhoea caused by these organisms was under-diagnosed (29).

EHEC could produce complications leading to haemolytic-uremic syndrome (HUS), a potentially fatal disorder marked by the destruction of red blood cells and kidney failure. EHEC has become a growing problem in the United States because of outbreaks caused by contaminated food. A particular type of EHEC, known as O157:H7 has been identified since 1982 in

undercooked hamburgers, unpasteurized milk, and apple juice. Between 2-7% of infections caused by O157:H7 develop into HUS. In United State, EHEC contamination is 20,000 infections and 250 deaths annually (30). A reported from Norway, serogroup of *E. coli* O103 was caused for nine of HUS cases and outbreak in 2006(31).

1.2. Escherichia coli.

E. coli is a gram-negative bacterium. The **genus** of *E. coli* has the specific ability to ferment glucose and lactose, but it usually does not produce H_2S. About 99% of *E. coli* **strains** are found to be indole test positive and belong to the **family** of *entero-bacteriaceae* and the **tribe** of *Escherichia* (32),(33). *E. coli* can be recovered easily from clinical specimens on general or selective media at 37°C under aerobic conditions, e.g. Mac-Conkey, eosin methylene-blue agar (34).

E. coli serotypes are specific O-group/H-antigen combinations. There are more than 700 antigenic types (serotypes) that are recognized by the presence of O, H, and K antigens (35). So far 170 different O antigens and at least 56 H antigens have been recognized.(5),(36)

E. coli inhabits the intestinal tract of humans and other animals. Most of them do not cause the disease (37). However, *E. coli* is a very **versatile** bacterium, and important subtypes of *E. coli* contain and express virulence factors that enable them to exhibit pathogenicity.

Pathogenic *E. coli* strains cause infections such as urinary tract infection (uropathogenic *E. coli*), sepsis/meningitis, and enteric/diarrhoea diseases (5).

DEC is classified base on pathogenic features with emphasis placed on the mechanisms of disease causation. The diagnostic techniques are based on **virulence factors**. DEC is classified as shown in ***annex5***

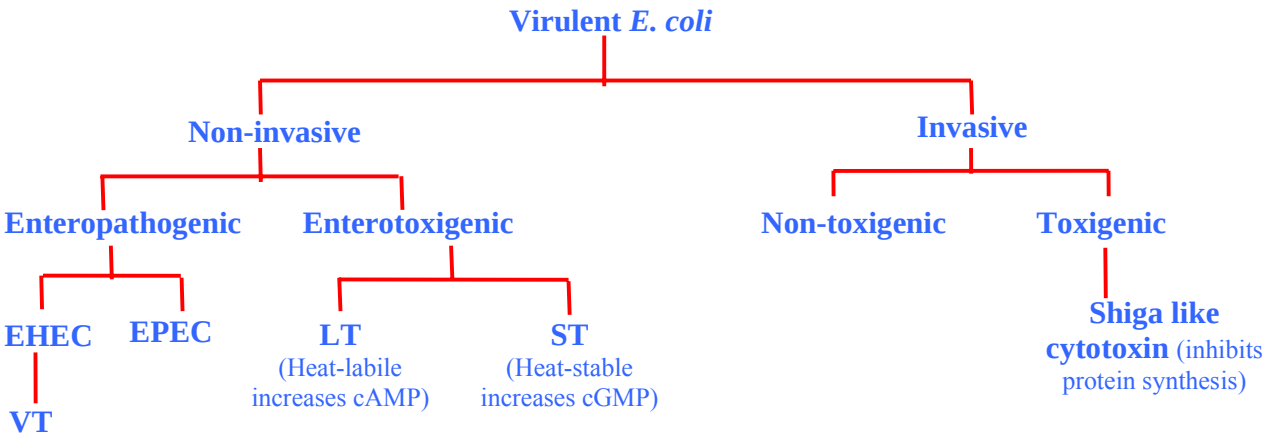

Figure 1: Virulence mechanisms of *E coli*. (39)

1.3. Epidemiology characteristically and transmission routes of diarrheagenic *E. coli*

The primary habitat of *E. coli* is the intestinal tract of humans and other warm-blooded animals. *E. coli* infections in humans are transmitted directly from animals, by person-to-person contact and via contaminated foods. Widespread faecal contamination of the environment (soil or water) by farm animals and, wild animals provides a continuing source of DEC in the agricultural environment and hence, in a wide variety of **raw foods**.

Domestic pets such as dogs and cats are potential carriers of *E. coli* including serogroups containing types pathogenic to humans, e.g. O55, O111, and O128. Other domesticated animals including cattle, pigs, sheep, especially the young ones and poultry carry *E. coli* as their commensal "normal" flora.

ETEC infection is transmitted by food and water contaminations (15;17;20). ETEC infection in developing countries is caused by fecal contamination of water and food sources. Warm and wet months are the most efficient for breeding ETEC infections (38). Heat Stable entero-toxin (ST) producing ETEC strains are usually responsible for the majority of the endemic cases (39;40) Although ETEC infection occurs most frequently among infants, immunologically naive adults are the most susceptible. Indeed, ETEC is the predominant etiologic agent causing traveler's diarrhea among adults from the developed world visiting areas where ETEC infection is found to be endemic (11-13;39;41). Studies suggest that 20 to 60% of such travelers experience diarrhea.

Typically, 20 to 40% of cases are caused by ETEC. Predictably, ETEC traveler's diarrhea occurs most commonly in warm and wet months as well as among first-time travelers to the developing world (11).

EPEC infection is primarily a disease of infants younger than 2 years (42). The correlation is strongest with infants younger than 6 months. Several outbreaks of diarrhea due to EPEC have been reported among healthy adults (43-46), presumably due to ingestion of a large inoculums from a common source. Sporadic disease has also been seen among adults with compromising factors (diabetic patients, the elderly) (42).

EAEC infection is related to persistent diarrhea (more than 14 days). It is more common in children (47-49). There are many reports of EAEC outbreaks (50;51). Patients in these outbreaks experienced vomiting and diarrhea, usually without fever. Persistent diarrhea occurs in a small number of patients. Outbreaks are associated with consumption of a restaurant meal, but a single source could be different to identify (52).

EIEC strains are probably often misidentified as *Shigella species* or nonpathogenic *E. coli* strains. Documented EIEC outbreaks are usually food-borne or water-borne (53-56), although person-to-person transmission also can take place (14). Endemic sporadic diseases can occur in certain areas, generally where *Shigella species* are found to be prevalent, but the epidemiological features may be different from those of *Shigella*.(57). The incidence of EIEC in developed countries is thought to be quite low, but occasional food-borne outbreaks had been reported.

EHEC can be transmitted by food and water and from person to person. Most cases are caused by ingestion of contaminated food, particularly those of bovine origin. The salient features of the EHEC epidemiology include a reservoir in the intestinal tract of cattle and other animals; transmission by a wide variety of food items, with beef being a major vehicle of infection; and a very low infectious dose, enabling high rates of attack and of person-to-person transmission The large outbreaks involving hundreds of individuals have gathered most attention, but sporadic EHEC infections comprise the major disease burden caused by this pathogen. EHEC is one of the most significant pathogens in developed countries. The *Shiga Toxin* producing *E. coli* (STEC) can be found in the fecal flora of a wide variety of animals including cattle, sheep, goats, pigs, cats, dogs, chickens, and gulls (58-61). *Shiga Toxin* producing *E. coli* strains are usually isolated from healthy animals but may be associated with an initial episode of diarrhea in young animals followed by asymptomatic colonization.

The main reservoir for the EHEC is the **intestines of healthy cattle**. EHEC does not cause clinical disease in adult cattle. EHEC is excreted in feces of infected cattle, humans and other infected animals. It can be transmitted by a number of routes: food-borne, water-borne, and person-to-person. Undercooked beef (i.e. hamburgers), cross contamination or fecal contamination of food or water and consumption of raw milk are among the most common sources of outbreaks.

The virulence of EHEC is such that only a few organisms (100–200) are necessary to cause disease. (62)

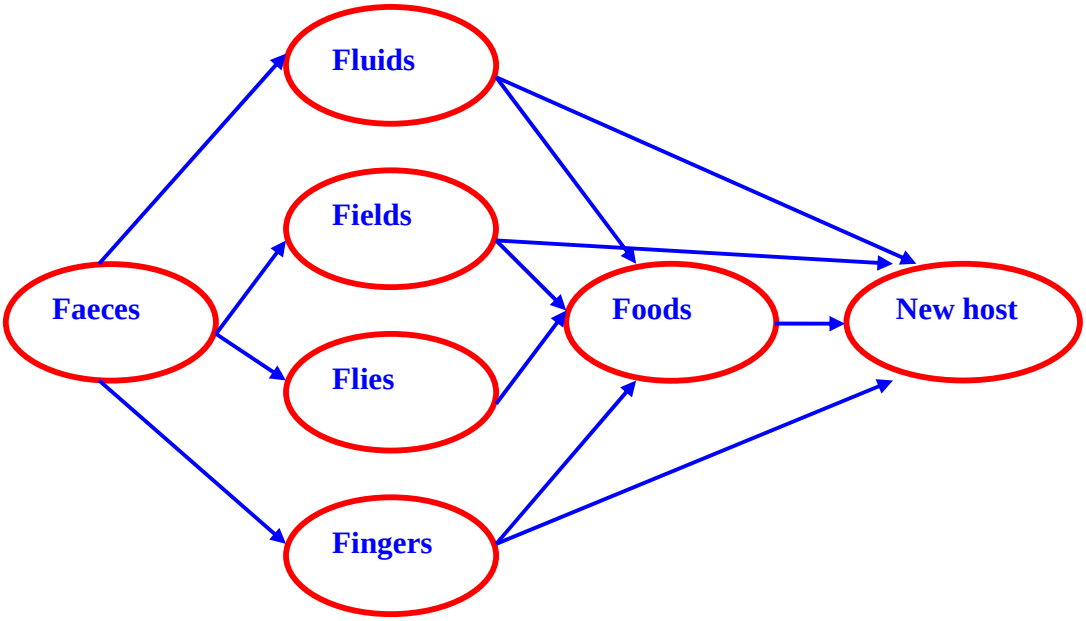

Figure 2: The F-diagram

1.4. Potential risk factors associate with transmission DEC disease:

Food hygiene related factors: Many studies have explored factors related to food hygiene associated with transmission of diarrhoeal diseases such as negligence of washing hands before eating or before preparing food (63), presence of many flies in the kitchen (64), presence of animals and pets in the food preparation area, (65) etc.

Drinking-water related factors: Some studies have found diarrheas long to be more associated with unsafe water source e.g. ponds, wells, rivers, lakes (66-71), distance to water source (72), low per-capita water used (73;74), use of wide-mouthed water vessels (75-79), obtaining water from vessels by dipping (80), not putting a lid on water storage vessels (81;82)

Sanitation and rubbish disposal factors: Some studies have shown that lack of latrine (83), unsanitary behavior in the disposal of stools (83), inadequate disposal of the feces and household refuse (84;85), visible feces on latrines floor (86) and sharing latrines (87) were strongly associated with increasing incidence of diarrhoeal diseases.

Socioeconomic factors: Some studies of socioeconomic factors as risk factors for diarrhea have found that poor status or poor living conditions (88;89) were associated with increasing diarrhoeal diseases.

Demographic factors: In some studies demographic factors such as age, sex, marital status, education, etc are associated with increasing incidence of diarrhoeal diseases. These are younger age (90-92), male gender, (84) low level of education (85), high number of siblings (89) and large households. (88).

Seasonal factors: The fecal-oral transmission route for diarrhoeal diseases is enhanced by the contamination or pollution of drinking water sources like the unprotected wells, rivers, ponds etc, by unsafe fecal disposal. The unhygienic practice of defecating in the bush may be a major source of ground water contamination. These fecal matters pollute the unprotected water source after being transported down slopes by run-off or overland flow. The run-off is largely experienced in the rainy seasons within the tropics, hence high level of pollution should occur in the rainy season in such regions.

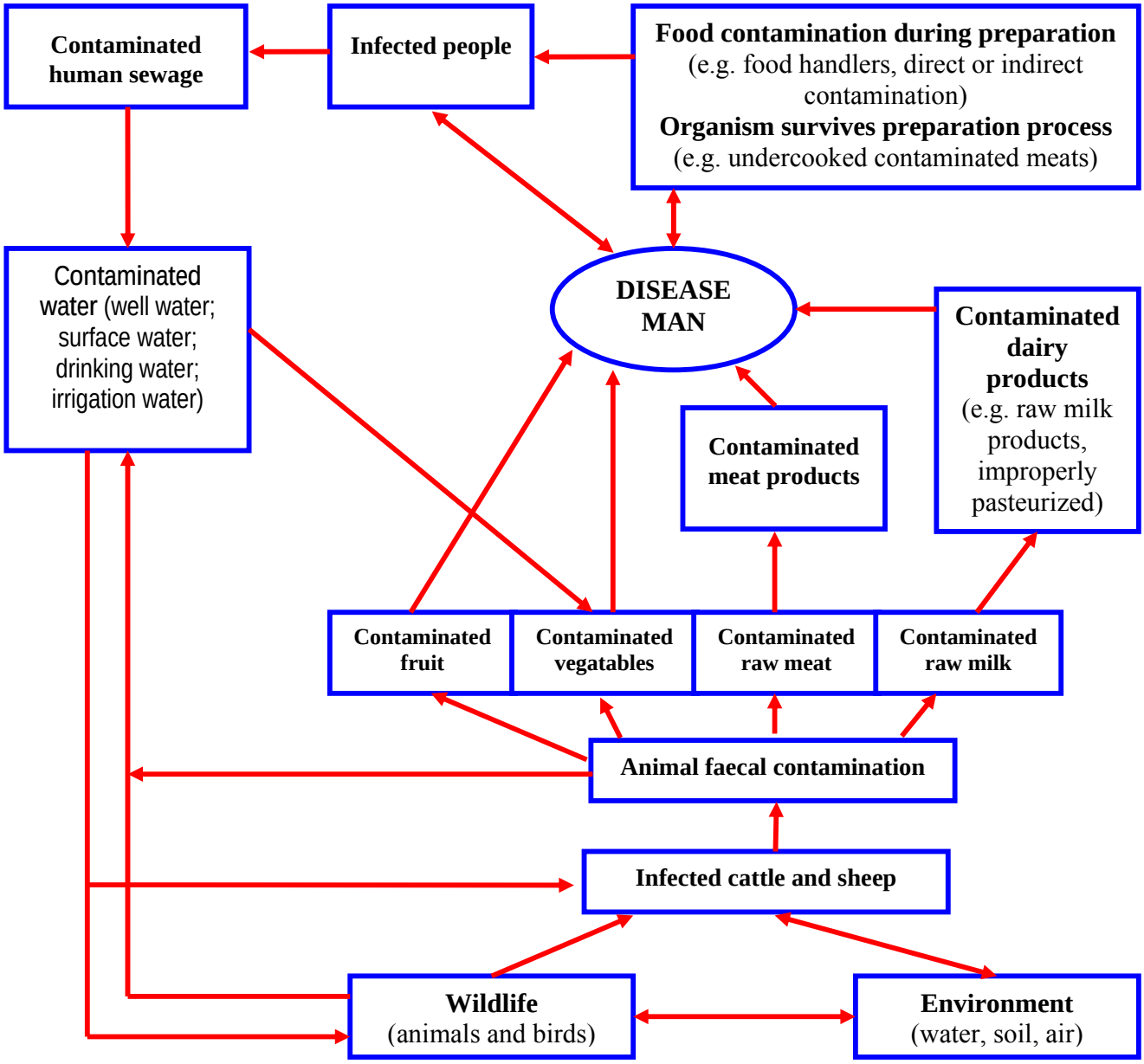

Figure 3: Modified version of the current main routes of transmission of DEC (1)

1.5. Clinical manifestations:

E. coli is a major enteric pathogen in developing countries and the different types of *E. coli* associated with enteric infections can be classified into five groups according to their virulence properties as follows:

EPEC strains in the past were associated with serious outbreaks of diarrhoea, and many clinical reports emphasize the severity of the diseases (93-95). They remain an important cause of

acute infantile diarrhoeal diseases in developing countries (8;9;94). Such occurrences are rare in adults.

The EIEC strains produce diseases resembling shigellosis among adults and children.

ETEC strains are a major cause of traveller's diarrhoea, and of infantile diarrhoeal diseases in the developing countries.

EHEC occur largely as a single serotype (O157:H7) causing sporadic cases and outbreaks of hemorrhagic colitis characterized by bloody diarrhoea. EHEC also may cause haemolytic uremic syndrome (HUS), a combination of haemolytic anaemia, thrombocytopenia, and acute renal failure.

EAEC strains exhibit a characteristic aggregative pattern of adherence to intestinaland produce persistent gastroenteritis and diarrhoea in infants and children in the developing countries.

1.6. Diagnosis:

The definitive diagnosis of diarrheagenic *E. coli* requires isolation from a specimen such as stool, rectal swab, vomit etc. The diagnosis should be suspected in case a patient presents with diarrhoea, abdominal pain, fever and bloody diarrhoea.

The definition of DEC has changed drastically in recent years with growing knowledge of the biology of host-parasite relationship.

For many years these organisms were defined only by O sero-groups. Subsequently this was refined to O:H serotypes. Now, sero-grouping by O antigens is not considered to be sufficient to identify a strain as diarrheagenic, because it does not correlate in most cases with the presence of virulence factors (96).

Currently, there are newer methods to diagnosis DEC such as Enzyme Linked Immunosorbent Assay (ELISA), latex agglutination and multiplex Polymerase Chain Reaction (PCR). ELISA and latex agglutination methods can be used to detect only some of the diarrheagenic *E. coli* pathotypes. But **multiplex PCR** with **several PCR primers** (depicted in table 2) are combined with the aim of detecting **several different diarrheagenic *E. coli*** pathotypes in a single reaction.

1.7. Treatment:

The most important first step in treatment of diarrhoea is to prevent dehydration. In severe cases, dehydration can be life threatening, particularly among children. Agitation may be an early symptom. Severe symptoms include restlessness and a weak pulse. Fluid replacement must use be done with solutions that contain the important minerals, potassium, sodium, and calcium.

Anti-motility agents provide prompt but temporary symptomatic relief by reducing muscle spasms in the gastrointestinal tract. Anti-motility agents should be discontinued if symptoms persist beyond 48 hours and should not be used at all under the following circumstances: i) in patients with high fever; and ii) when there is blood in the stool.

Antibiotics are generally effective for treating diarrhoea patients who develop the disease within eight-hours before start of treatment, with three or more loosely stools per day, and especially if associated with nausea, vomiting, abdominal cramps, fever or presence of blood in the stools. Because antibiotics are prescription drugs, travellers at risk should obtain them before they depart and should receive directions for self-treatment while abroad. Antibiotics should not be used for mere symptoms as nausea and vomiting if diarrhoea is not an associated condition.

Although self-treatment is generally safe, a doctor should be consulted for any child with diarrhoeal disease and for adult patients who develop fever or bloody diarrhoea. Antibiotics are generally not useful for diarrhoeal diseases in developed nations, since such cases are likely to be caused by viruses.

In general, patients take one tablet of ofloxacin every 12 hours for five days. Taking a single dose of an antibiotic such as ofloxacin, together with an anti-motility agent, often provides relief within 24 hours for many patients. Other antibiotics used for diarrhoeal diseases include ciprofloxacin, norfloxacin, and azithromycin.

1.8. Prevention and control:

Avoiding foods and beverages that could be contaminated with bacteria can prevent DEC infections. Insufficiently cooked and uncooked foods are associated with DEC infections. High-risk foods are raw fruits and vegetables (e.g., salads), raw seafood or under-cooked meat or poultry, unpasteurized dairy products, food from street vendors, and untreated water (including ice cuber) in areas lacking adequate chlorination.

In developing countries, food can be made safe to eat by thoroughly cooking it and by keeping it hot until eaten. Person ingesting fruits and vegetables should peel them beforehand.

Water used for drinking (including brushing teeth) or for washing food in these countries should be bottled, boiled, or chemically treated with iodine, chlorine or another disinfectant. Hand-washing with soap and water with prevents contamination of food and beverages with DEC and prevent transmission from person to person. Bismuth subsalicylate preparations (1 ounce of liquid or two 262.5-mg tablets taken four times daily) can reduce the risk of becoming infected with ETEC and other common bacteria that cause diarrhoeal diseases. Persons with kidney diseases should consult a physician before taking medications with large amounts of salicylates

Vaccines for ETEC are in clinical testing, but none are currently available.(97)

Taking antibiotics to prevent ETEC infection is not recommended. Pregnant women and persons with weakened immune systems should talk with their health care provider if they are travelling to areas known to be at high risk for ETEC exposure.

DEC disease is best controlled by preventing transmission and by stressing the importance of breast-feeding of infants, especially where ETEC is endemic. The best treatment is oral fluid and electrolyte replacement (intravenous in severe cases). Antibiotics are only recommended in special cases because a wider use will lead to an increased burden of antibiotic-resistant pathogenic *E coli* and of other virulent life-threatening entero-pathogens.

Intervention of the fecal-oral transmission cycle is most effective in institutional situations.

1.9. Country profile: Viet Nam

Background:

Figure 4: The map of Viet Nam

Viet Nam is located in the centre of Southeast Asia. The country has bordered with three countries (China, Laos and Cambodia) and two seas (South China Sea and Pacific Ocean). The country has a land area of 329,560 square kilometres.

According to the last national census from 1999, 79.6 millions inhabitants are living in Viet Nam, but following the statistical record in 2003, there are over 81 million people with population growth an annual around 1.29 %. There are 56 ethnic groups; among them 85% belong to the Kinh ethnic group. Other groups are distributed in the mountainous and forest areas from the north to the south. Around 20% of the population live in urban areas.

Viet Nam is located in both the tropical and the temperate zone. It is characterised by strong monsoon influences, but has a considerable amount of sun, and a high rate of rainfall and humidity. The annual average temperature ranges from 22^{o}C to 27^{o}C. The rainfall, which is generally

abundant over the entire country range from an average of 2,000 mm in the north to 1,700 mm in the south with a small pocket of 700 mm in Thuan Hai province. There are two distinguishable seasons in the whole country: the cold season which lasts from November to April and the hot season which lasts from May to October. The difference in temperature between the two seasons in Southern Viet Nam is almost unnoticeable, averaging at 3^{o}C. The most noticeable variations are found in the Northern provinces where differences of 12^{o}C are observed. There are essentially four distinct seasons, most evident in the Northern provinces. In Hanoi, the average temperature is 23^{o}C. In Ho Chi Minh City it is 26^{o}C.

Health sector:

The health care system in Viet Nam is organized along a four-tiered pyramid. At the top of the pyramid is the Ministry of Health, which is the main national authority in the health sector and together with the provincial, district and commune people's committees, formulates and executes the health policies and programs for the whole the country (98).

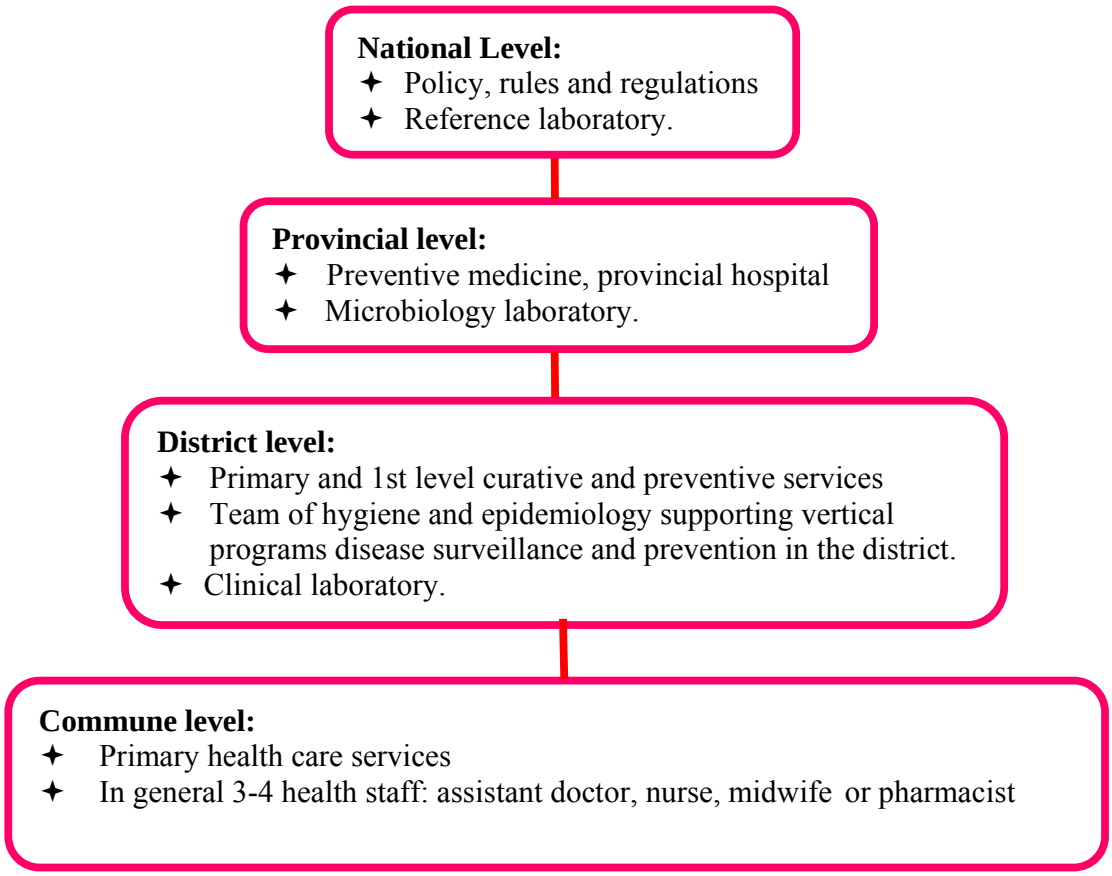

Figure 5: Health system in Viet Nam

The country is divided into 63 provinces, which are relatively self-supporting. Each province has its own health service with general hospitals, clinics and pharmacies. A province is divided into districts and a district has many communes. Each level is responsible for the distribution of health stations, general hospitals, and pharmaceutical stores. Viet Nam has many private clinics and pharmacies authorised by the Ministry of Health, especially in big cities such as Ha Noi, Ho Chi Minh City, etc and in the towns of the provinces (99).

In spite of being one of the poor countries in Asia, Viet Nam's overall state of health, as measured by conventional indicators, is much better than would be expected for a country with its considerably low income per capita. It is obvious that Viet Nam’s achievements in reducing infant mortality are impressive and have few parallels in the world. The infant mortality rate of Viet Nam is already among the low rates in the Asia-pacific region (100).

Table 1: Infant mortality rate of some Asia countries (by year 2004)

Country	Infant mortality rate (per 1000)
Cambodia	73.67
Indonesia	36.82
India	57.92
Laos	87.06
Malaysia	18.35
Myanmar	68.78
Philippines	24.24
Thailand	21.14
Viet Nam	**29.88**

Viet Nam has experienced an epidemiological transition during the last 20 years with a major decline in the share of communicable disease in mortality and morbidity. While communicable diseases accounted for 50-56% of mortality and morbidity in 1976, the corresponding figure was 27% by 1997. This shift reflects the success of communicable disease control programs, particularly the expanded program of immunization, which has dramatically reduced the incidence of vaccine-preventable diseases in Viet Nam.

Despite the decline in their incidence, infectious diseases continue to remain a major public health problem in Viet Nam. In 2002, for instance, malaria, diarrhoea, and respiratory tuberculosis were among the ten leading cause of admissions at public hospitals together accounting for nearly 600,000 cases (101).

Viet Nam's economy:

Viet Nam is a poor, densely populated country that has had to recover from the ravages of war, the loss of financial support from the old Soviet bloc and the rigidities of a centrally planned economy. Substantial progress was achieved from 1986 to 1996 in moving forward from an extremely low starting point. Growth in Gross Domestic Product (GDP) averaged around 9% per year from 1993 to 1997. The 1997 Asian financial crisis highlighted the problems and strength in the Vietnamese economy. Rather than prompting reform, it reaffirmed the government's belief that shifting to a market-oriented economy would lead to disaster. GDP growth of 8.5% in 1997 fell to 6% in 1998 and 5% in 1999. Growth then rose to 6% to 7% in 2000-02, even against the background of global recession. These numbers mask some major difficulties in economic performance. Many domestic industries, including coal, cement, steel, and paper, have reported large stockpiles of inventory and tough competition from more efficient foreign producers. Since the Party elected new leadership in 2001, Vietnamese authorities have reaffirmed their commitment to economic liberalization and have moved to implement the structural reforms needed to modernize the economy and to produce more competitive, export-driven industries. The US-Vietnam Bilateral Trade Agreement entered into force near the end of 2001 and is expected to significantly increase Viet Nam's exports to the US. The US is assisting Viet Nam with implementing the legal and structural reforms called for in the agreement. The Gross National Product (GNP) per capita income is 432US$ (102).

Diarrheagenic *E. coli* in Viet Nam:

In Viet Nam, different studies have shown that diarrheagenic *E. coli* is the main cause of morbidity and mortality in children. A study of children under 5 years of age in Ha Noi found a prevalence of DEC at 22.5% among 587 fecal samples from children with diarrhoeal diseases (2). They found EAEC in 11.6%, EIEC in 2.0%, EPEC in 6.6%, and ETEC in 2.2%.

A study of Ryukyu University – Japan and NIHE-Viet Nam showed that Shiga-toxin producing *Escherichia coli* (STEC) could be isolated from feces of patients and cows in Viet Nam in 2002 (103).

A study from Can Tho University showed that in 169 swine faeces sample, there were 6% of STEC and 3% of EAEC (104).

There are only a few studies focused on diarrheagenic *E. coli* in Viet Nam. A study showed that most diarrheagenic *E. coli* strains isolated from Viet Nam in 2004 were resistant to antibiotic community used, but were sensitive to ofloxacin, ciprofloxacin, norfloxacin and erythromycin(105).

Currently, several hospital laboratories in Viet Nam identify pathogenic *E. coli* by biochemical methods and serogrouping of O antigens focused on EPEC. Probably a great number of DEC cases are misdiagnosed or not detected.

Study area:

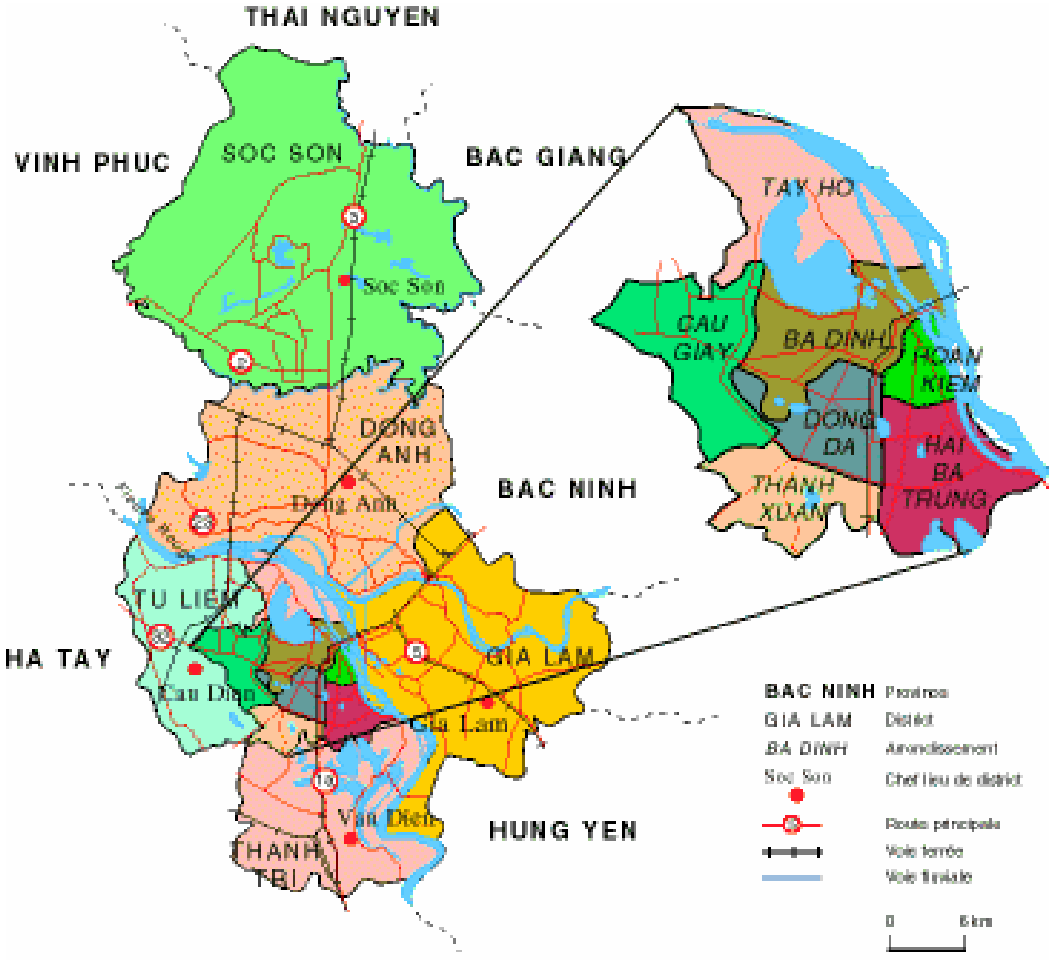

Figure 6: Ha Noi map

❖ **Long Bien and Gia Lam District:**

Gia Lam District is a suburban district in the northeastern corner of Ha Noi City beyond the Red River. Gia Lam borders with two provinces (Bac Ninh, Hung Yen) and four districts in Ha Noi (Dong Anh, Hoan Kiem, Thanh Tri and Long Bien). There are about 278,000 inhabitants living in this district. The density is 2572-persons/km^2.

Long Bien district is an urban district in the northeastern corner of Ha Noi. This district just established in 2005 by division of Gia Lam District. This district is closer to the centre of Ha Noi and Red River. Long Bien borderes with four districts in Ha Noi (Thanh Tri, Hoan Kiem, Dong

Anh and Gia Lam). There are about 190,000 inhabitants living in this district. The density is 2832-persons/km^2

The annual average temperature is 23.6^o C with its minimum of 4^o C and maximum 39.4^o C. Drinking water is piped in some wards near the centre of Ha Noi. Most people in the rural area use water from wells, ponds, rivers and rain-water. There are only a few houses with standard latrine facilities. Gia Lam and Long Bien districts constitute one of three main areas providing food for the centre of Ha Noi. The main products are vegetables, fruits and meat.

- **Duc Giang Hospital:**

Duc Giang Hospital was established in 1973 with a total of 110 staff including 32 doctors and 300 beds, offering health care mostly to people in Gia Lam and Long Bien district. At this particular hospital, there are at least 300 turnovers of patients receiving medical treatment over a single working day. More than 1,000 patients with diarrhoeal diseases visit the Duc Giang Hospital every year.

Chapter II: Research question and Objectives of the study

2.1. Research questions:

What factors are associated with diarrhoeal diseases and DEC disease in Long Bien and Gia Lam districts?

2.2. Objectives:

- To identify DEC in diarrhoea patients in Duc Giang Hospital.
- To identify risk factors associated with diarrhoeal disease and DEC disease in Long Bien and Gia Lam Districts.

Chapter III: Methodology

3.1. Variables and definitions used in this study:

Two types of variables were used in this study, namely dependent and independent variables.

Dependent variables:

The study had one dependent variable which is DEC disease. DEC disease was defined as three or more, loose, liquid or watery stools or at least one bloody loose stool within 24 hours and positive culture with an *E. coli* strain that carried at least one of these genes: *stx, eae, est, elt, ipaH, aggR*.

Independent variables:

The independent variables in the study were regarded as the potential risk factors for transmission DEC disease based on a literature review, including demographic, socioeconomic factors, sanitation-, hygiene-, food-, occupation- and water-related factors.

- **Education level:** This variable was defined according to attendance to school (illiteracy, completion of primary level, secondary grade, comprehensive school education and university graduations). The education was considered as significant information, as illiteracy often related to poverty, living under unhygienic condition and possibly associated with the poor access to the health education messages.
- **Occupation:** According to the literature review, breeders, health professionals, microbiologists, or sewage workers were considered to have a higher risk of contracting DEC than the general population. Therefore, this variable was investigated.
- **Contact with diarrhoea patients:** This factor was described as a risk factor for diarrhoeal disease. Because of the faecal – oral mode of the transmission, close contact with a patient, when taking care of him in the hospital or at home, living in the same house or visiting the patients house could involve a higher risk of contracting the disease.
- **Population migration:** People who move or travel somewhere, could be exposed and fall sick after they are back to this area.
- **Food habits:** Food habits such as eating in the street or consumption of some foods (raw fish, shellfish, salad and ice-cream) have been implicated in food-born communicable diseases.
- **Food-related hygiene:** Improper food storage in the kitchen or in the dining room might lead to contamination of foods through flies and multiplication of bacteria.

- **Personal hygiene practices:** Habits regarding washing hands before eating or preparing food and after going to toilet might also increase the risk of enteric infection.
- **Water supply:** The different types of water supply and now drinking water was collected are important factors in transmission.
- **Sanitation condition (environment):** In most endemic areas, poor sanitation, open sewage, disposal of faeces directly in the environment, open latrines are likely to contribute to the contamination of water sources.
- **Family economic level:** The economic level was considered as affecting the living conditions and changing the risk patterns of contracting diseases.

3.2. Study design: A hospital-based case-control study

Case-control studies are often used to identify risk factors associated with diseases. In a case-control study patients who have developed a disease are identified and their past exposure to suspected aetiological factors was compared with that of controls or referents that do not have the disease.

A case-control design was applied to identify risk factors associated with of diarrhoeal disease and DEC diseases.

The starting point of a case-control study is the identification of cases. This requires a suitable and precise case definition. In addition, particular attention was needed in the selection process in order to avoid that bias did arise from the way in which cases were selected. Two controls per case were commonly recruited. Controls were randomly selected from diarrhoea patients who proved negative with DEC test and non-diarrhoea patients.

A case-control study allows estimating odds ratios (OR). Allowance was made for potential confounding factors by measuring them and making appropriate adjustment in analysis.

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Selection of cases and controls:

Cases: A case was defined as patients with diarrhoeal diseases who were found to be positive for virulence genes of *E. coli* (figure 1) in the multiplex PCR test.

Controls: For each case, select two ‘controls 1’ were selected (diarrhoea negative for DEC) and one ‘control 2’ (non-diarrhoea)

The first control was a person admitted to the hospital with the same symptoms as the case but negative with virulence gene of *E. coli*

The second control was randomly chosen in non-diarrhoea patients in Duc Giang Hospital.
Both controls were matched by age and sex.

Sample size and selection:

In this study, convenience sampling was used to collect samples. All diarrhoea patients in Duc Giang Hospital were subjects to stool specimens to identify cases. The stool specimen's collection was stopped when we had identified 62 cases.

3.3. Microbiology methods:

Collect samples: Each stool specimen was collected in a special container with Cary-Blair transport medium, kept at 4^{o}C, and transferred to the microbiology laboratory within 24 hours for analysis.

Stool cultures: Fecal samples collected in Cary-Blair transport medium were cultured on the surface of Sorbitol-MacConkey Agar (SMAC) followed by overnight incubation at 37^{o}C. Separate colonies were subjects to biochemical tests. Strains suspected of being *E. coli* were tested with multiplex PCR to identify virulence genes of *E. coli.*

Multiplex PCR methods to identify the virulence gene of *E. coli*:

- *E. coli* strains were cultured in 2 ml of Luria-Bertani broth (1% tryptone, 0.5% yeast extract, 0.5% NaCl) and incubated overnight at 37°C with shaking.
- **Extract DNA:** Thirty-six microliters of broth culture was add to 4µl of 10X Tris-EDTA buffer (100 mM Tris-HCl, 10 mM EDTA, pH 8.3), and 60µl of 2X proteinase K buffer (100 mM KCl, 20 mM Tris-HCl, 5 mM MgCl2, 1% Tween 20, 800µg of proteinase K/ml, pH 8.3) was add. After incubation for 90 min at 56°C and 10 min at 95°C, the sample will centrifuge at 10,000 X g for 1 min, and the supernatant was use as DNA template.
- **PCR mixture reaction:** Having confirmed the specificity of each primer set by single PCR, we combined six primer sets in different ratios and tested the control strains in several PCR cycling protocols. The optimized protocol was carry out with a 50-µl mixture containing 10 mM Tris-HCl (pH 8.3); 50 mM KCl; 0.1% Triton X-100; 1.5 mM MgCl2; 2.5 U of Taq DNA polymerase (Toyobo, Osaka, Japan); 0.2 mM deoxynucleoside triphosphate; a 0.125 µM concentration (each) of primers SK1, SK2, ipaIII, and ipaIV; a 0.25 µM concentration (each) of primers VTcom-u, VTcom-d, LTL, LTR, aggRks1, and aggRkas2; a 0.5 µM concentration (each) of primers AL65 and AL125; and 5 µl of the DNA template.

- The PCR program was 95°C for 1 min, 52°C for 1 min, and 72°C for 1 min, for 30 cycles, and 72°C for 10 min.
- PCR products was electrophoreses on a 2.5% agarose gel (AmpliSize; Bio-Rad Laboratories), stain with ethidium bromide, and visualized by UV transillumination. The buffer in the electrophoresis chamber and in the agarose gel was 0.5X Tris-borate-EDTA (11).
- All the virulence gene of *E. coli* had single PCR methods for reconfirmation.

Table 2: PCR primers used in this study:

	Primer	**Designation Sequence (5'to 3')**	**Target gene**	**Amplicon size (bp)**	**Reference**
EPEC	SK1	CCCGAATTCGGCACAAGCATAAGC	*eae*	881	(106)
	SK2	CCCGGATCCGTCTCGCCAGTATTCG			
STEC	VTcom-u	GAGCGAAATAATTTATATGTG	*stx*	518	(107)
	VTcom-d	TGATGATGGCAATTCAGTAT			
ETEC	AL65	TTAATAGCACCCGGTACAAGCAGG	*est*	147	(108)
	AL125	CCTGACTCTTCAAAAGAGAAAATTAC			
	LTL	TCTCTATGTGCATACGGAGC	*elt*	322	(109)
	LTR	CCATACTGATTGCCGCAAT			
EIEC	ipaIII	GTTCCTTGACCGCCTTTCCGATACCGTC	*ipaH*	619	(110)
	ipaIV	GCCGGTCAGCCACCCTCTGAGAGTAC			
EAEC	aggRks1	GTATACACAAAAGAAGGAAGC	*aggR*	254	(111)
	aggRkas2	ACAGAATCGTCAGCATCAGC			

3.4. Data collection methods:

Training skills to the interviewers:

Five interviewers were selected, among them two from the Enteric Pathogens Laboratory at NIHE and three medical doctors from the Duc Giang Hospital. The interviewers were instructed how to ask the questions and how to report exactly what the respondents answered. The interviewers practised together to ensure a standardised way of collecting information.

Laboratory training:

Training of laboratory technicians was under taken in Duc Giang Hospital. This was done to ensure that stool sample collection and storage were done in compliance with the standard protocol. We also trained the laboratory technicians in Enteric Pathogens Laboratory-NIHE, to improve their skills to identify virulence genes of *E. coli* and to following the standard protocol.

Data collection tool:

The questionnaire: To avoid ambiguous answers, a questionnaire with clear and simple questions was designed. The questionnaire was pre-tested and had both closed and open-ended questions. The questionnaire had several sections: a section on demographic, a section on possible source of infection, a section on family economic condition, a section on clinical data and a section on laboratory data.

The questionnaire was developed in English language (***annex 2***) and translated into Vietnamese, the only language for communication in the district.

Data collection techniques

Interviews:

- Face-to-face interviews based on the questionnaire were conducted on patients in Duc Giang Hospital where cases and controls recruited into the study.
- Participants were visited at their hospital bed.
- Patients and controls were enrolled after they had given informed consent. (Annex 1)
- Most of questions had multiple choices. (Annex 2)

Piloting/pre-testing:

The pilot study was conducted with the first ten suspected cases with both collection and stool specimens and interview following the questionnaire. After the pilot study, some adjustments were made to the questionnaire (see results)

Data handling, analysis and processing:

All data collected were validated at the end of each day by cross-checking to see if all information needed was actually collected and to assess for consistency in recording. All the information was summarized and each individual's data set was given a number to avoid mixing up the files.

The principal investigator has the full responsibility of data analysis. Preliminary analysis of the qualitative data was carried out manually during the process of data collection, while for the quantitative data it was done at the end of the data collection. The analyzed data was described as variables and analysed to answer the research questions.

Finally, data collected were entered into a computer for analysis. Software Package for Social Sciences (SPSS) version 13.0 was used for the analysis of the data. All variables considered potential risk factors were tested. The difference between cases and controls were evaluated by estimating the odds ratios (OR).

Univariate analysis was performed to identify factors associated with diarrhoea with the statistical significance set at the level $P < 0.05$.

Conditional logistic regression analyses were performed to identify which of these potential risk factors remained independently associated with diarrhoeal disease and DEC disease. Attributable fractions were calculated by using the multivariate odds ratio and percentage exposed among the cases.

Strengths and weaknesses of the data collection tools/ approaches:

The strength of a structured questionnaire is that there is an element of accuracy of measurements which enhances quantification, and controls for bias. The results obtained from such interviews can usually be generalized to a larger population. They were also easy to administer, manage and could be quickly analysed statistically.

However, there are weaknesses related to this method as little is known about what people actually do and how they experience, perceive and evaluate things.

Some weaknesses of this method were inherent in the demand for personal interaction. Cooperation of the participants is very essential as some of them may be uncomfortable sharing with the researcher most of what he/she is looking for. In many instances, language may be a barrier so the interviewer may not be able to evoke long narratives from the respondents. Last, but not the least, a lot of data was generated during the whole process of interview which increases the risk for clerical errors.

3.5. Research team:

In collaboration between Enteric Pathogens Laboratory, NIHE, and Duc Giang Hospital, a research team was established including the principal researcher, 3 researchers from Enteric Pathogens Laboratory, NIHE and 5 staffs from Duc Giang Hospital.

- Enteric Pathogens Laboratory, NIHE.

Responsibilities:

- Training interviewers
- Training staff working in the laboratory
- Regular quality checks on interview skills and work in the laboratory.
- Identify of *E. coli* strains in the reference laboratory at NIHE.
- Conduct staff seminars for reporting progress, discussion of problems, maintaining good morale.
- Data collection.

- Duc Giang Hospital:

Responsibilities:

- Collected and transported stool samples of suspected patients from the hospital to NIHE
- Selection of controls in the hospital.
- Conducted interviews in the hospital.

3.6. Ethical considerations:

Scientific merit: The results from the study will provide answers to the questions being addressed. Furthermore, results of this study could help health authorities to adapt a policy to control this disease.

Informed consent: Cases or controls in this study were given standard information of the purpose and procedure of the study. They were also told that oral consent only is requested as many of them were illiterate. They could freely accept or refuse to be interviewed without any consequence for their treatment on care in the hospital.

Confidentiality of the records, names, and addresses: The research team guaranteed the confidentiality for the information volunteers provided.
Informed written permission (memorandum of understanding) from authorities in the hospital and in the communities were obtained before starting the study.
The project was reviewed for ethical clearance by the Ministry of Health in Viet Nam.

Feedback results: The results of the study will be reported to the population studied to the hospital staff, to the local community leaders, to the local and national health officials and to the funding agencies that supported the study. There are plans to publish results of this study in a peer reviewed journal

3.7. Time table.

June 2005	
13-17	- Working with health authorities of Duc Giang Hospital. - Working with Enteric Pathogens Laboratory, NIHE - Design and pilot test record form and questionnaires. - Make arrangement for staff, training, equipment, transport, finance, and accommodation. - Training interview skills for interviewers. - Training for staff working in the laboratory in Duc Giang Hospital. - Draw up daily work plan for all staffs.
21 - 31	- Pilot test all organisational details. - Modification of questionnaires as necessary. - Arrange for laboratory procedures. - Conduct staff seminars for reporting progress, discussion about problem, maintaining morale.
July 2005	- Data collection - Working in laboratory
August 2005	- Data collection - Working in laboratory
September 2005	- Data collection - Working in laboratory.
October 2005	- Data collection - Working in laboratory.
November 2005	- Data collection - Working in laboratory.
December 2005	- Data collection - Working in laboratory.
January 2006	- Entry data into the computer. - Data compilation and analysis
February - June 2006	- Writing thesis. - Thesis defence

Chapter IV: Results of study

4.1. Some on-site adjustments to the study:

- The hospital's name was changed to Duc Giang Hospital. It mainly serves Long Bien and Gia Lam districts. Long Bien district is a new district; it was a part of Gia Lam district previous year.
- The study was conducted in 2 departments: Paediatrics Department for patients who are children under 15 years old and Enterology Department for patients over 16 years old.
- The pilot study was conducted with the first 20 diarrhoeal patients admitted to the hospital.
- The original questionnaire has adapted for two purposes: one used for patients in Paediatrics Department and the other used for patients in Enterology Department.

4.2. Characteristic of the study sample:

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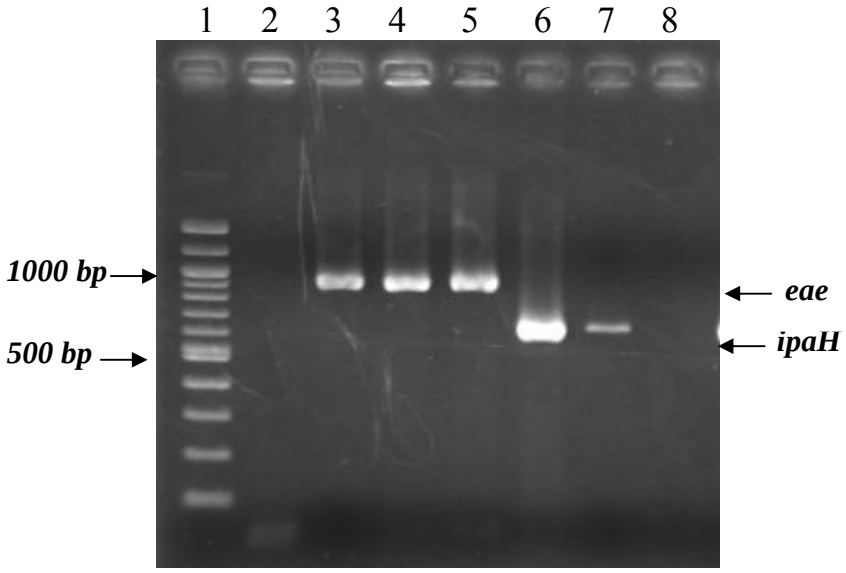
Table 3: Distribution of patients and cases by department in Duc Giang hospital.

	Patients admitted	Patients out of study	Patients in Pilot study	Patients in study	DEC cases
Paediatrics Department	314	12	13	289	56
Enterology Department	102	9	7	86	6
Total	416	21	20	375	62

There was a total of 416 diarrhoeal patients admitted to the hospital. 21 of them were excluded from the study because they did not meet the criteria as described in chapter 3. For example, they came from other districts or they had only one day of treatment in hospital, etc.
Stool specimens were collected from 289 diarrhoeal patients in Paediatrics departments and 86 diarrhoeal patients in Enterology Departments for the detection the DEC. These patients were also interviewed to explore the risk factor associated with transmission DEC disease.

Laboratory results:



Lane 1: Marker
Lane 2: Negative control
Lane 3: Positive control
Lane 4, 5: EPEC ***(eae: 881 bp)***
Lane 6, 7: EIEC ***(ipaH: 619 bp)***
Lane 8: Negative sample

Figure 7: Multiplex PCR from DEC from stool samples

During the study period, 62 DEC strains were found from the total of 375 suspected cases of DEC according to our registered case definition. All DEC cases were identified by multiplex PCR and confirmed by single PCR.

The pilot test was done with first 20 diarrhoeal patients from 17 – 25 June 2005, but no DEC strains were found. From 1 July 2005 –25 December 2006, the total of 62 DEC strains were isolated from 375 stool samples from diarrhoea patients. Figure 7 shows one of the results of multiplex PCR for DEC from stool samples in diarrhoeal patients.

We did not find any *Vibrio cholera* or *Salmonella* species. However, 12 *shigella* strains (3.2%) including 7 strains *Shigella flexneri* and 5 strains *Shigella sonnei* were found from the specimens.

Table 4: DEC distribution by categorization

Categorization of *E. coli*	Gene	n	%
EAEC	***aggR***	30	48.4
ETEC	***elt, est***	16	25.8
EPEC	***eae***	13	21.0
EIEC	***ipaH***	3	4.8
Total		62	

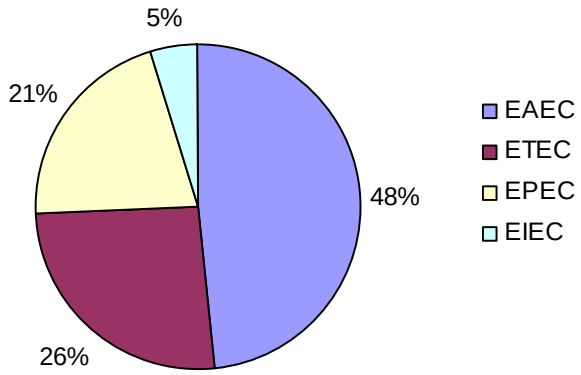


Figure 8: DEC distribution by categorization

The prevalence of DEC in diarrhea patient was 62/375 (16.5%), 52/270 (19.3%) in children under 5; 4/19 (21.1%) in children between 6-15 years old; and 6/86 (6.9%) in adults. PCR assays detected 30/62 EAEC (48.4%), 13/62 EPEC (21%), 16/62 ETEC (25.8%), and 3/62 EIEC (4.8%) in diarrhea patients. No EHEC was found in any of the specimens.

Geographical distribution:

Table 5: Cases distribution by district.

District	Number of case	Population	Attack Rate (%)
Long Bien	19	190,743	0.010
Gia Lam	43	214,512	0.020
Total	62	405,255	0.015

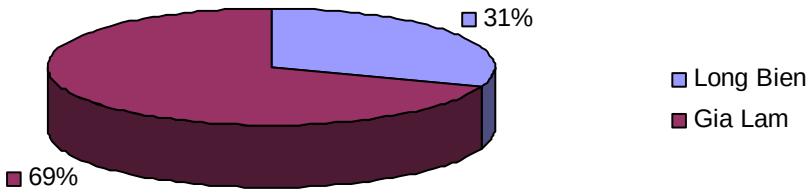


Figure 9: Distribution of cases by district

The first case was reported on July 3, 2005 (week 26) and the last case was reported on December 21, 2005 (week 51). 19 DEC cases (30.6%) lived in Long Bien district and 43 cases (69.4%) lived in Gia Lam District.

We identified 124 diarrhoea controls and 62 Non diarrhoea control matched with cases by age and sex. None of them had a positive for DEC disease.

Sex distribution:

Table 6: Sex distribution by districts

District	Male		Female	
	n	(%)	n	(%)
Long bien	10	30.3	9	31.0
Gia lam	23	69.7	20	69.0
Total	33	53.2	29	46.8

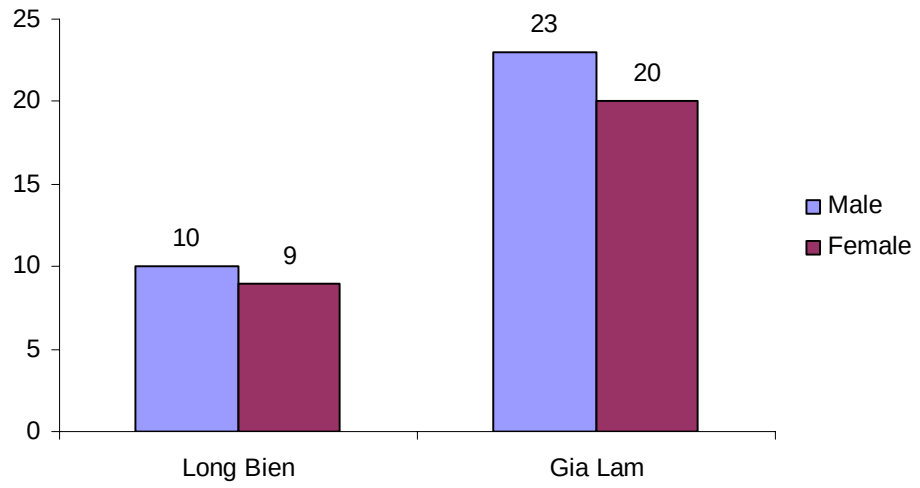


Figure 10: Sex distribution by districts

The sex ratio (male/female) was 1.14. In total, there were 33 (53.2%) males and 29 (46.8%) females in our study.

Age distribution

Table 7: Age and Sex distribution

Ages group	*Sex*		*Cases*	
	Male	**Female**	**n**	**%**
< 5 years	26	23	49	79.0
5 - 9 years	4	3	7	11.3
45 - 49 years	2	0	2	3.2
50 - 54 years	0	1	1	1.6
65 - 69 years	0	1	1	1.6
70 - 74 years	0	1	1	1.6
75 – 79 years	1	0	1	1.6
Total	**33**	**29**	**62**	

Most of the cases were among children under 5 years old as described in table 7. There were no cases found in the age groups 10-14, 15-19, 20-24, 25-29, 30-34, 35-39, 40-44, 55-59 and 60-64 year olds. In other age groups, there was very few case.

Clinical manifestation and treatment received:

Table 8: Clinical symptoms of DEC patients

Clinical symptoms	EAEC (30)	EPEC (13)	ETEC (16)	EIEC (3)
Fever (n, %)	18 (60)	7 (53.85)	10 (62.5)	3 (100)
Headache (n, %)	7 (23.33)	1 (7.69)	1 (6.25)	0
Vomiting (n, %)	15 (50)	10 (76.92)	10 (62.5)	2 (66.7)
Dehydration				
Mild (n, %)	10 (33.33)	2 (15.38)	6 (37.5)	2 (66.7)
Moderate (n, %)	20 (66.66)	11 (84.61)	10 (62.5)	1 (33.33)
Diarrhoea less than 7 days (n, %)	8 (26.67)	9 (69.23)	8 (50)	0
Diarrhoea more than 7 days (n, %)	22 (73.33)	4 (30.77)	8 (50)	3 (100)
Blood with stool (n, %)	4 (13.33)	3 (23.08)	5 (31.25)	1 (33.33)

There were 37/62 (59.7%) cases with vomiting symptom and 38/62 (61.3%) cases with fever. Only 9/62 (14.5%) cases were reported to have headache. 25/62 (40.3%) cases reported that they had a diarrhoeal period less than 7 days, 36/62 (58.1%) cases reported diarrhoea between 8-14 days. 1/62 (1.6%) cases had diarrhoea more than two weeks.

There were 9/62 (14.5%) cases who had antibiotics before being admitted to hospital. 5 cases took chloramphenicol, 3 cases took sulfamethoxazole + trimethoprim, and 1 case took phenobarbital.

For treatment in the hospital, all cases were given antibiotics following the prescription of the doctor. 56/62 (90.3%) cases used one kind of antibiotics, 6/62 (9.7%) cases used two kinds of antibiotics. 10/62 cases used sulfamethoxazole + trimethoprim, 38/62 cases used nalidixic acid, 8/62 cases used spiramicine, 3/62 cases used nalidixic acid – cefuroxime, and 1/62 case used nalidixic acid – spiramicine.

There was no death case reported.

Risk factors for diarrhoea disease and DEC disease:

Table 9: Significant risk factors in univariate analysis among patients groups

The risk factors associated with diarrhoeal disease and DEC disease	Diarrhoea & non-diarrhoea	DEC & non-diarrhoea
Having contact with diarrhoeal patients	-	+
Occupations :		
Food vendor	+	+
Farmer/Fisher/Breeder	+	+
Farmer/Fisher/Breeder/Food vendor	+	+
Often eating outside:		
1-2 times/month	-	-
1-2 times/week	-	+
Where to eating foods outside last week:		
In the Pub	-	+
In the street	-	+
Eating outside last week		+
Foods eating last week:		
Salad/fruits	-	-
Half cooked beef	-	-
Roasted meat	-	-
Source water for domestic use/purpose:		
Well water	+	+
Pond water	+	+

(+) : mean significant (OR> 1; P< 0.05; 95% CI without value 1)

(-) : mean not significant (OR< 1; P> 0.05)

Table 9 (continue): Significant risk factors in univariate analysis between patients groups

The risk factors associated with diarrhoea disease and DEC disease	Diarrhoea & non-diarrhoea	DEC & non-diarrhoea
Washing hands before eating:		
Sometimes	+	+
Never	+	+
Negligence	+	+
Washing hands after goes to toilet:		
Sometimes	+	-
Never	+	+
Negligence	+	
Where to keeping foods:		
In the kitchen without a cover	+	+
In food tray with a cover	+	+
Keeping food outside the fridge	+	+
Household garbage:		
Open surrounding	-	-
Dispose of waste water		
Garden	-	-
Income		
Low	+	+

(+) : mean significant (OR> 1; P< 0.05; 95% CI without value 1)

(-) : mean not significant (OR< 1; P> 0.05)

4.3. Result of comparison between the groups of diarrhoeal disease & non-diarrhoeal disease:

- **Univariate analysis:**

Table 10: Prevalence of risk factors associated with diarrhoeal disease in univariate analysis

Potential risk factors	Diarrhoea (Cases = 186)	Non-diarrhoea (Controls = 62)	Matched Odd Ratio (OR)	95% Confidence Intervals (CI)	P-value
Education:					
Bachelors	10/186 (5.4%)	5/62 (8.1%)	Ref.		
Senior secondary school	97/107 (90.7%)	36/41 (87.8%)	1.4	0.4-4.2	0.607
Junior secondary school	60/70(85.7%)	16/21 (76.2%)	1.9	0.6-6.3	0.302
Illiteracy /Primary	19/29 (65.5%)	5/10 (50%)	1.9	0.4-8.2	0.384
Contact with diarrhoea patient:					
No	147/186 (79%)	51/62 (82.3%)	Ref.		
Don't know	9/156 (5.8%)	6/57 (10.5%)	0.5	0.2 – 1.5	0.230
Yes	30/177 (16.9%)	5/56 (8.9%)	2.1	0.8-5.7	0.143
If yes, where:					
In hospital	11/186 (5.9%)	3/62 (4.8%)	Ref.		
In neighbourhood	13/24(54.2%)	2/5 (40%)	1.7	0.2 – 12.6	0.564
Occupations:					
Civil servant/teacher/Food Industry/ Health-worker/Student:	86/186(46.2%)	41/62 (66.1%)	Ref.		
Food vendor:	**14/100(14%)**	**1/42 (2.4%)**	**6.7**	**0.9-52.5**	**0.04**
Unemployment:	10/96(10.4%)	3/44 (6.8%)	1.5	0.4-6.1	0.496
Farmer/Fisher/Breeder :	**76/162(46.9%)**	**17/58(29.3%)**	**2.1**	**1.1-4.1**	**0.020**
Farmer/Fisher/Breeder/Food vendor	**90/176(51.1%)**	**18/59(30.5%)**	**2.3**	**1.3-4-5**	**0.006**
History of travel:					
No	168/186 (90.3%)	59/62 (95.2%)	Ref.		
Yes	12/124(9.7%)	3/62 (4.8%)	2.1	0.6-7.4	0.236
Often eating outside:					
Never	116/186 (62.4%)	44/62 (71%)	Ref.		
Rarely	41/157 (26.1%)	13/57 (22.8%)	1.2	0.6-2.4	0.622
1-2 times/month	14/130 (10.8%)	3/47 (6.4%)	1.8	0.5-6.5	0.382
1-2 times/week	15/131 (11.5%)	2/46 (12.6%)	2.9	0.6-13.0	0.160

Table 10 (cont.): Prevalence of risk factors associated with diarrhoeal disease in univariate analysis

Potential risk factors	Diarrhoea (Cases = 186)	Non-diarrhoea (Controls = 62)	Matched Odd Ratio (OR)	95% Confidence Intervals (CI)	P-value
Eating outside last week:					
No	138/186 (74.2%)	53/62 (85.5%)	Ref.		
In the pub/restaurant	18/156 (11.5%)	4/57 (7%)	1.7	0.6-5.3	0.337
In the street	30/168(17.9%)	5/58 (8.6%)	2.3	0.9-6.3	0.094
Foods eating last week:					
Shellfish/seafood	40/186(21.5%)	8/62 (12.9%)	Ref.		
Ice-cream manufactured by	10/50 (20%)	6/14 (42.9%)	0.3	0.09-1.2	0.081
Home made ice-cream	4/44(9.1%)	1/9 (11.1%)	0.8	0.08-8.1	0.850
Fresh milk	86/126 (68.3%)	14/22 (63.6%)	1.2	0.5-3.2	0.669
Local beer	16/56 (28.6%)	5/13 (38.5%)	0.6	0.2-2.3	0.485
Salad/fruits	30/70 (42.9%)	7/15(46.7%)	0.6	0.2-1.9	0.331
Half-cooked beef	20/60 (33.3%)	3/11 (27.3%)	1.3	0.3-5.6	0.693
Roasted meat	29/69 (42%)	5/13 (38.5%)	1.2	0.3-3.9	0.811
Source of water for domestic use:					
Municipal water supply	66/124 (37.1%)	53/62 (85.5%)	Ref.		
Rainwater	21/87 (24.1%)	11/64 (17.2%)	1.5	0.7-3.5	0.302
Well	**119/185 (64.3%)**	**37/91 (41.8%)**	**2.6**	**1.5-4.3**	**<0.001**
Pond	**17/83 (20.5%)**	**3/56 (5.4%)**	**4.6**	**1.3-16.4**	**0.013**
Washing hand before eating:					
Usually	39/186 (21%)	44/62 (71%)	Ref.		
Sometimes	**123/162(75.9%)**	**14/58 (24.1%)**	**9.9**	**4.9-20.0**	**<0.001**
Never	**24/63 (38.1%)**	**4/48 (8.3%)**	**6.8**	**2.2-21.2**	**<0.001**
Negligence	**147/186(79%)**	**18/62 (29%)**	**9.2**	**4.8-17.7**	**<0.001**
Washing hand after goes to toilet:					
Usually	78/186 (41.9%)	43/62 (69.4%)	Ref.		
Sometimes	**88/166 (53%)**	**16/59 (27.1%)**	**3.0**	**1.6-5.8**	**0.001**
Never	**20/98 (20.4%)**	**3/46 (6.5%)**	**3.7**	**1.0-13.1**	**0.034**
Negligence	**108/186 (58.1%)**	**19/62(30.6%)**	**3.1**	**1.7-5.8**	**<0.001**
Take a bath:					
Private bathroom	167/186 (89.8%)	58/62 (93.5%)	Ref.		
Sharing bathroom	17/184 (9.2%)	4/62 (6.5%)	1.5	0.5-4.6	0.497

Table 10 (cont.): Prevalence of risk factors associated with diarrhoeal disease in univariate analysis

Potential risk factors	Diarrhoea (Cases = 186)	Non-diarrhoea (Controls = 62)	Matched Odd Ratio (OR)	95% Confidence Intervals (CI)	P-value
Keeping food:					
In refrigerator	66/186 (35.5%)	41/62 (66.1%)	Ref.		
In food tray with a cover	**60/126 (47.6%)**	**12/53 (22.6%)**	**3.1**	**1.5-6.5**	**0.002**
In the kitchen without a cover	**60/126 (47.6%)**	**9/50 (18%)**	**4.1**	**1.9-9.2**	**<0.001**
Keeping food outside the fridge	**120/186 (64.5%)**	**21/62 (33.9%)**	**3.6**	**1.9-6.5**	**<0.001**
Type of latrines:					
Dry latrine:	**35/186 (18.8%)**	**4/62 (6.5%)**	**3.4**	**1.2-9.9**	**0.021**
Dry latrine:	35/186 (18.8%)	4/62 (6.5%)	Ref.		
Modern toilet:	118/153 (77.1%)	52/56 (92.9%)	0.3	0.09-0.8	0.010
Wet latrine :	17/5 (32.7%)	4/8 (50%)	0.5	0.1-2.2	0.339
Excrete directly into the field/ fishpond:	16/51 (31.4%)	2/6 (33.3%)	0.9	0.2-5.5	0.922
Household garbage:					
Rubbish pit	168/186 (90.3%)	58/62 (93.5%)	Ref.		
Open surrounding	18/186 (9.7%)	4/62 (6.5%)	1.6	0.5-4.8	0.249
Dispose of waste water:					
Sewage system	153/186(82.3%)	56/62 (90.3%)	Ref.		
Pond	17/170 (8.4%)	3/59 (5.1%)	2.1	0.6-7.4	0.249
Garden	13/1166 (4.4%)	3/59 (5.1%)	1.6	0.4-5.8	0.481
Kinds of livestock:					
Dogs	67/186 (36%)	23/62 (37.1%)	Ref.		
Cats	11/78 (14.1%)	4/27 (14.8%)	0.9	0.3-3.3	0.927
Chickens	45/112 (40.2%)	9/32 (28.1%)	1.7	0.7-4.0	0.214
Pigs	45/112(40.2%)	12/35 (34.3%)	1.3	0.6-2.9	0.532
Cattle	14/81 (17.3%)	2/25 (8%)	2.4	0.5-11.4	0.257
People in the house:					
Under 5	148/186 (79.6%)	52/62 (83.9%)	Ref		
Over 6	38/186 (20.4%)	10/62 (16.1%)	1.3	0.6-2.9	0.458
Income:					
High	39/186 (21%)	20/62 (32.3%)	Ref.		
Average	103/142 (72.5%)	34/54 (63%)	1.6	0.8-3.0	0.192
Low	**44/83 (53%)**	**8/28 (28.6%)**	**2.8**	**1.1-7.1**	**0.025**

The factor **civil servant/teacher/food industry/health-worker/student** in categorization of **occupation** was used as a reference for the comparisons among different factors. The factors **food vendor** (OR: 6.7, 95%CI: 0.9-52.5, P/F: 0.04/0.04) and **farmer/fisher/breeder** (OR: 2.1, 95%CI: 1.1-4.1, P: 0.02) were strongly associated with the diarrhoeal disease. When these factors were combined to be factor **farmer/fisher/breeder/food vendor**, it was found to be strongly associated with the diarrhoea disease (OR = 2.3, 95%CI 1.3 – 4.5, P = 0.006).

The factor **municipal water supply** in categorization of **source of water for domestic use** was used as a reference for the comparisons among different factors. The factors **well water** (OR: 2.6, 95%CI: 1.5-4.3, P: <0.001) and **pond water** (OR: 4.6, 95%CI: 1.3-16.4, P/F: 0.013/0.014) were strongly associated with the diarrhoeal disease.

The factor **negligence washing hand before eating** in categorization of **washing hands before eating** was used as a reference for the comparisons among different factors. The factors **sometime washing hand before eating** (OR: 9.9, 95%CI: 4.9-20.0, P: <0.001) and **never washing hands before eating** (OR: 6.8, 95%CI: 2.2-21.2, P/F: <0.001/<0.001) were significantly associated with the diarrhoeal disease. When these factors were combined to be factor **negligence washing hands before eating,** it was also found to be significantly associated with the disease (OR = 9.2, 95%CI 4.8 – 17.7, P = <0.001).

The factor **usually washing hands after going to toilet** in categorization of **washing hands after going to the toilet** was used as a reference for the comparisons among different factors. The factors **sometime washing hands after going to toilet** (OR: 3.0, 95%CI: 1.6-5.8, P: 0.001) and **never washing hands after going to toilet** (OR: 3.7, 95%CI: 1.0-13.1, P/F: 0.034/0.049) were significantly associated with the diarrhoea disease. When these factors were combined into factor **negligence washing hands after going to toilet,** it was also significantly associated with the disease (OR = 3.1, 95%CI 1.7 – 5.8, P = <0.001).

The factor **keeping food in fridge** in categorization of **keeping food** was used as a reference for the comparisons among different factors. The factors **keeping food in food tray with a cover** (OR: 3.1, 95%CI: 1.5-6.5, P: 0.002) and **keeping food in the kitchen without a cover** (OR: 4.1, 95%CI: 1.9-9.2, P: <0.001) were significantly associated with the diarrhoeal disease. When these factors were combined into one factor called **keeping food outside the fridge,** it was also significantly associated with the diarrhoea disease (OR = 3.6, 95%CI 1.9 – 6.5, P = <0.001).

The factor **dry latrine** in categorization of **type of latrine** (OR: 3.4, 95%CI: 1.2-9.9, P: 0.021) was strongly associated with diarrhoeal disease.

The factor **high income** in categorization of **income** was used as a reference for the comparisons among different factors. The factor **low income** (OR: 2.8, 95%CI: 1.1-7.1, P: 0.025) was found to be strongly associated with the diarrhoeal disease.

Multivariate analysis:

Risk factors that were significantly associated with increasing diarrhoeal disease in the univariate analysis were chosen to do multivariate analysis.

Variables were considered for the model:

- Farmer/fisher/breeder/food vendor
- Well water
- Pond water
- Negligence washing hands before eating
- Negligence washing hands after goes to toilet
- Keeping food outside the fridge
- Dry latrine
- Low income

Table 11: Result of the multivariate analysis (I)

Potential risk factors	Matched Odd Ratio (OR)	95% Confidence Intervals (CI)	P-value
Farmer/fisher/breeder/food vendor	1.6	0.7-3.4	0.2
Well water	0.8	0.4-1.5	0.4
Pond water	2.2	0.5-10.2	0.3
Negligence washing hands before eating	**13.3**	**5.1-34.6**	**<0.001**
Negligence washing hands after goes to toilet	0.5	0.2-1.4	0.2
Keeping food outside the fridge	**2.8**	**1.4-5.9**	**0.006**
Dry latrine	1.6	0.5-5.5	0.5
Low income	1.4	0.5-3.9	0.5

In the multivariate analysis for diarrhoea group & non-diarrhoea group, conditional logistic regression was used. Two among 8 exposures shown in table 11 **(negligence washing hands before eating** and **keeping food outside the fridge)** increased the risk of diarrhoeal disease. Other exposures, in spite of being risk factors according univariate analysis, appeared to remain independently associated with diarrhoeal disease when they were included in the model of conditional logistic regression.

4.4. Result of comparison between the groups of DEC disease & non-diarrhoeal disease:

- **Univariate analysis:**

Table 12: Prevalence of risk factors associated with DEC disease in univariate analysis (I)

Potential risk factors	DEC (Cases = 62)	Non-diarrhoea (Controls = 62)	Matched Odd Ratio (OR)	95% Confidence Intervals (CI)	P-value
Education:					
Bachelors	3/62 (4.8%)	5/62(8.1%)	Ref.		
Senior secondary school	31/34 (91.2%)	36/41 (87.8%)	1.4	0.3-6.5	0.638
Junior secondary school	15/18 (83.3%)	16/21 (76.2%)	1.6	0.3-7.7	0.582
Primary school	8/11 (72.7%)	4/9 (44.4%)	3.3	0.5-21.6	0.199
Illiteracy	5/8(62.5%)	1/6 (16.7%)	8.3	0.6-110.0	0.086
Contact with diarrhoea patient:					
No	43/62 (69.4%)	51/62 (82.3%)	Ref.		
Don't know	4/47 (8.5%)	6/57 (10.5%)	0.8	0.2 – 3.0	0.729
Yes	**15/58 (25.9%)**	**5/56 (8.9%)**	**3.6**	**1.2-10.6**	**0.017**
If yes, where:					
In hospital	6/62 (9.7%)	3/62 (4.8%)	Ref.		
In neighbourhood	8/9(88.9%)	5/6 (83.3%)	2.0	0.3 – 16.0	0.510
Occupations:					
Civil servant/teacher/food Industry/ health-worker/student:	26/62(41.9%)	41/62 (66.1%)	Ref.		
Food vendor:	**9/35 (25.7%)**	**1/42 (2.4%)**	**14.2**	**1.7-118.7**	**0.002**
Unemployment:	2/28 (7.1%)	3/44 (6.8%)	1.1	0.2-6.7	0.958
Farmer/fisher/breeder:	**25/51(49%)**	**17/58(29.3%)**	**2.3**	**1.2-5.1**	**0.035**
Farmer/fisher/breeder/food vendor:	**34/60(56.7%)**	**18/59(30.5%)**	**3.0**	**1.4-6.3**	**0.004**
History of travel:					
No	56/62 (90.3%)	59/62 (95.2%)	Ref.		
Yes	6/62(9.7%)	3/62 (4.8%)	2.1	0.5-8.8	0.299
Often eating outside:					
Never	32/62(51.6%)	44/62 (71%)	Ref.		
Rarely	12/44 (27.3%)	13/57 (22.8%)	1.3	0.5-3.1	0.606
1-2 times/month	9/41 (22%)	3/47 (6.4%)	4.1	1.0-16.5	0.034
1-2 times/week	**9/41 (22%)**	**2/46 (12.6%)**	**6.2**	**1.3-30.6**	**0.014**

Table 12 (cont.): Prevalence of risk factors associated with DEC disease in univariate analysis (I)

Potential risk factors	DEC (Cases = 62)	Non-diarrhoea (Controls = 62)	Matched Odd Ratio (OR)	95% Confidence Intervals (CI)	P-value
Eating outside last week:					
No	35/62 (56.5%)	53/62 (85.5%)	Ref.		
In the pub/restaurant	**10/45 (22.2%)**	**4/57 (7%)**	**3.8**	**1.1-13.0**	**0.027**
In the street	**17/52 (32.7%)**	**5/58 (8.6%)**	**5.2**	**1.7-15.2**	**0.002**
Eat outside	**27/62 (43.5%)**	**9/62 (14.5%)**	**4.5**	**1.9-10.8**	**<0.001**
Foods eating last week:					
Shellfish/Seafood	13/62 (21%)	8/62 (12.9%)	Ref.		
Ice-cream manufactured by	4/17 (23.5%)	6/14 (42.9%)	0.4	0.09-1.9	0.252
Home made ice-cream	1/14 (7.1%)	1/9 (11.1%)	0.6	0.03-11.3	0.742
Fresh milk	39/52 (75%)	14/22 (63.6%)	1.7	0.6-5.0	0.322
Local beer	9/22 (40.9%)	5/13 (38.5%)	1.1	0.3-4.5	0.886
Salad/fruit	17/30 (56.7%)	7/15(46.7%)	1.5	0.4-5.2	0.526
Half-cooked beef	14/27 (51.9%)	3/11 (27.3%)	2.9	0.6-13.2	0.157
Roasted meat	22/35 (62.9%)	5/13 (38.5%)	2.7	0.7-10.1	0.130
Source of water for domestic use:					
Municipally supplying water	20/62 (32.3%)	53/62 (85.5%)	Ref.		
Rainwater	9/29 (31%)	11/64 (17.2%)	2.2	0.8-6.0	0.132
Well	**41/61 (67.2%)**	**38/91 (41.8%)**	**2.9**	**1.5-5.6**	**0.002**
Pond	**11/31 (35.5%)**	**3/56 (5.4%)**	**9.7**	**2.5-38.5**	**<0.001**
Washing hands before eating:					
Usually	13/62 (21%)	44/62 (71%)	Ref.		
Sometimes	**40/53 (75.5%)**	**14/58 (24.1%)**	**9.6**	**4.1-23.0**	**<0.001**
Never	**9/22 (40.9%)**	**4/48 (8.3%)**	**7.6**	**2.0-28.8**	**0.001**
Negligence	**49/62 (79%)**	**18/62 (29%)**	**9.2**	**4.1-21.0**	**<0.001**
Washing hands after go to toilet:					
Usually	31/62 (50%)	43/62 (69.4%)	Ref.		
Sometimes	22/53 (41.5%)	16/59 (27.1%)	1.9	0.9-4.2	0.108
Never	9/40 (22.5%)	3/46 (6.5%)	4.2	1.0-16.6	0.033
Take a bath:					
Private bathroom	52/62 (83.9%)	58/62 (93.5%)	Ref.		
Sharing bathroom	9/61 (14.8%)	4/62 (6.5%)	2.5	0.7-8.6	0.134
River/pond					

Table 12 (cont.): Prevalence of risk factors associated with DEC disease in univariate analysis (I)

Potential risk factors	DEC (Cases = 62)	Non-diarrhoea (Controls = 62)	Matched Odd Ratio (OR)	95% Confidence Intervals (CI)	P-value
Keeping food:					
In refrigerator	20/62 (32.3%)	41/62 (66.1%)	Ref.		
In food tray with a cover	**19/39 (48.7%)**	**12/53 (22.6%)**	**3.3**	**1.3-8.0**	**0.009**
In the kitchen without a cover	**23/43 (53.5%)**	**9/50 (18%)**	**5.2**	**2.1-13.4**	**<0.001**
Keeping food outside the fridge	**42/62 (67.7%)**	**21/62 (33.9%)**	**4.1**	**1.9-8.7**	**<0.001**
Type of latrines:					
Dry latrine:	12/62 (19.4%)	4/62 (6.5%)	Ref.		
Modern toilet:	36/48 (75%)	52/56 (92.9%)	0.2	0.07-0.8	0.012
Wet latrine :	7/19 (36.8%)	4/8 (50%)	0.6	0.1-3.1	0.525
Excrete directly into the field/ fishponds:	7/19 (36.8%)	2/6 (33.3%)	1.2	0.2-8.1	0.876
Household garbage:					
Rubbish pit	52/62 (83.9%)	58/62 (93.5%)	Ref.		
Open surrounding	10/62 (16.1%)	4/62 (6.5%)	2.8	0.8-9.4	0.089
Dispose of waste water:					
Sewage system	47/62 (75.8%)	109/124	Ref.		
Pond	7/54 (13%)	(87.9%)	2.8	0.7-11.4	0.141
Garden	8/55 (14.5%)	3/59 (5.1%)	3.2	0.8-12.6	0.087
Kinds of livestock:					
Dogs	25/62 (40.3%)	23/62 (37.1%)	Ref.		
Cats	3/28 (10.7%)	4/27 (14.8%)	0.7	0.1-3.4	0.648
Chickens	19/44 (43.2%)	9/32 (28.1%)	1.9	0.7-5.2	0.179
Pigs	23/48 (47.9%)	12/35 (34.3%)	1.8	0.7-4.3	0.214
Cattle	9/34 (26.5%)	2/25 (8%)	4.1	0.8-21.2	0.072
People in the house:					
Under 5	53/62 (85.5%)	52/62 (83.9%)	Ref		
Over 6	9/62 (14.5%)	10/62 (16.1%)	0.9	0.3-2.4	0.803
Income:					
High	10/62 (16.1%)	20/62 (32.3%)	Ref.		
Average	36/46 (78.3%)	34/54 (63%)	2.1	0.9-5.2	0.096
Low	**16/26 (61.5%)**	**8/28 (28.6%)**	**4.0**	**1.3-12.5**	**0.015**

The factor **no contact with diarrhoea patients** was used as a reference for the comparisons among different factors. The factor **having contact with diarrhoea patients** (OR: 3.6, 95%CI: 1.2-10.6, P: 0.017) was significantly associated with the DEC disease.

The factor **civil servant/teacher/food Industry/health-worker/student** was used as a reference for the comparisons among different factors. The factors **food vendor** (OR: 14.2, 95%CI: 1.7-118.7, P/F: 0.002/0.004) and **farmer/fisher/breeder** (OR: 2.3, 95%CI: 1.2-5.1, P: 0.035) were strongly associated with the DEC disease. When these factors were combined into one factor named **farmer/fisher/breeder/food vendor**, it was also strongly associated with the disease (OR = 3.0, 95%CI 1.4 – 6.3, P = 0.004).

The factor **never eating outside** in categorization of **often eating outside** was used as a reference for the comparisons among different factors. The factor **eating outside 1-2 times/week** (OR: 6.2, 95%CI: 1.3-30.6, P/F: 0.014/0.021) was strongly associated with the DEC disease.

The factor **no eating outside last week** in categorization of **eating outside last week** was used as a reference for the comparisons among different factors. The factor **eating outside last week in the pub/restaurant** (OR: 3.8, 95%CI: 1.1-13.0, P/F: 0.027/0.041) and e**ating outside last week in the street** (OR: 5.2, 95%CI: 1.7-15.2, P/F: 0.002/0.002) were strongly associated with the DEC disease. When these factors were combined to be the factor **eating outside last week,** it was also strongly associated with the DEC disease (OR = 4.5, 95%CI 1.9 – 10.8, P = <0.001).

The factor **municipally water supply** in categorization of **source water for domestic use** was used as a reference for the comparisons among different factors. The factor **well water** (OR: 2.9, 95%CI: 1.5-5.6, P: 0.002) and **pond water** (OR: 9.7, 95%CI: 2.5-38.5, P/F: <0.001/0.001) were strongly associated with the DEC disease.

The factor **usually washing hands before eating** in categorization of **washing hands before eating** was used as a reference for the comparisons among different factors. The factor **sometime washing hands before eating** (OR: 9.6, 95%CI: 4.1-23.0, P: <0.001) and **never washing hands before eating** (OR: 7.6, 95%CI: 2.0-28.8, P/F: 0.001/0.002) were strongly associated with the DEC disease. When these factors were combined to be the factor **negligence washing hands before eating,** it was also strongly associated with the DEC disease (OR = 9.2, 95%CI 4.1 – 21.1, P = <0.001).

The factor **keeping food in refrigerator** in categorization of **keeping food** was used as a reference for the comparisons among different factors. The factors **keeping food in food tray with a**

cover (OR: 3.3, 95%CI: 1.3-8.0, P: 0.009) and **keeping food in the kitchen without a cover** (OR: 5.2, 95%CI: 2.1-13.4, P: <0.001) were strongly associated with the disease. When these factors were combined to be the factor **keeping food outside the fridge,** it was also strongly associated with the DEC disease (OR = 4.1, 95%CI 1.9 – 8.7, P = <0.001).

The factor **high income** in categorization of **income** was used as a reference for the comparisons among different factors. The factor **low income** (OR: 4.0, 95%CI: 1.3-12.5, P: 0.015) was found to be strongly associated with DEC disease.

- **Multivariate analysis:**

The risk factors that were significantly associated with diarrhoeal disease in the univariate analysis were chosen to do multivariate analysis of risk factors for DEC disease in comparing between groups of DEC disease and non-diarrhoeal disease.

Variables were considered for the model:

- Have contact with diarrhoeal patient
- Farmer/fisher/breeder/food vendor
- Eating outside 1-2 times/week
- Eating outside last week
- Well water
- Pond water
- Negligence washing hands before eating
- Keeping food outside the fridge
- Low income

Table 13: Result of the multivariate analysis (II)

Potential risk factors	Matched Odd Ratio (OR)	95% Confidence Intervals (CI)	P-value
Having contact with diarrhoeal patient	**6.1**	**1.4-26.6**	**0.02**
Farmer/fisher/breeder/food vendor	2.1	0.7-5.8	0.2
Eating outside 1-2 times/week	3.0	0.4-25.0	0.3
Eating outside last week	2.7	0.9-8.4	0.09
Well water	1.0	0.4-2.6	0.9
Pond water	4.0	0.5-29.4	0.2
Negligence washing hands before eating	**7.8**	**3.0-20.3**	**<0.001**
Keeping food outside the fridge	**3.7**	**1.3-10.3**	**0.012**
Low income	1.3	0.4-4.5	0.7

In the multivariate analysis for comparing between DEC group & non-diarrhoea group, conditional logistic regression was used. Three among 9 exposures (**contact with diarrhoea patient**, **negligence washing hands before eating** and **keeping food outside the fridge**) increased the risk of DEC disease as shown in table 13. Other exposures identified as risk factors in the univariate analysis. when being included in the model of conditional logistic regression, these factors did not remain independently associated with diarrhoeal disease.

4.5. Result of comparison between the groups of DEC disease & diarrhoea non-DEC disease:

- **Univariate analysis:**

Table 14: Prevalence of risk factors associated with DEC disease in univariate analysis (II)

Potential risk factors	DEC (Cases = 62)	Diarrhoea non DEC (Controls = 124)	Matched Odd Ratio (OR)	95% Confidence Intervals (CI)	P-value
Education:					
Bachelors	3/62 (4.8%)	7/124(5.6%)	Ref.		
Senior secondary school	31/34 (92.1%)	66/73(90.4%)	1.1	0.3-4.5	0.899
Junior secondary school	15/18 (83.3%)	45/52 (86.5%)	0.8	0.2-3.4	0.738
Primary school	8/11 (72.7%)	5/12 (41.7%)	3.7	0.6-21.6	0.133
Illiteracy	5/8 (62.5%)	1/8 (12.5%)	11.7	0.9-147.6	0.039
Contact with diarrhoea patient:					
No	43/62 (69.4%)	104/124 (83.9%)	Ref.		
Don't know	4/47 (8.5%)	5/109 (4.6%)	1.9	0.5 – 7.6	0.335
Yes	**15/58 (25.9%)**	**15/119 (12.6%)**	**2.4**	**1.1-5.4**	**0.027**
If yes, where to contact:					
In the school	1/62 (1.6%)	1/124 (0.8%)	Ref.		
In hospital	6/7 (85.7%)	5/6 (83.3%)	1.2	0.06 – 24.5	0.906
In neighbourhood	8/9(88.9%)	5/6 (83.3%)	1.6	0.08 – 31.8	0.576
Occupations:					
Civil servant/teacher/food Industry/health-worker/student:	26/62(41.9%)	60/124(48.4%)	Ref.		
Food vendor:	**9/35(25.7%)**	**5/65(7.7%)**	**4.2**	**1.3-13.6**	**0.013**
Unemployment:	2/28(7.1%)	8/68(11.8%)	0.6	0.1-2.9	0.50
Farmer/fisher/breeder :	25/51(49%)	51/111(45.9%)	1.1	0.6-2.2	0.716
History of travel:					
No	56/62 (90.3%)	112/124 (90.3%)	Ref.		
Yes	6/62(9.7%)	12/124(9.7%)	1.0	0.4-2.8	1.0
Often eating outside:					
Never	32/62(51.6%)	84/124 (67.7%)	Ref.		
Rarely	12/44 (27.3%)	29/113 (25.7%)	1.1	0.5-2.4	0.837
1-2 times/month	**9/41 (22%)**	**5/89 (5.6%)**	**4.7**	**1.5-15.2**	**0.005**
1-2 times/week	**9/41 (22%)**	**6/90 (6.7%)**	**3.9**	**1.3-11.9**	**0.011**
More than 1-2 times/month	**18/62 (22%)**	**11/124 (8.9%)**	**4.2**	**1.8-9.6**	**<0.001**

Table 14 (cont.): Prevalence of risk factors associated with DEC disease in univariate analysis (II)

Potential risk factors	DEC (Cases = 62)	Diarrhoea non DEC (Controls = 124)	Matched Odd Ratio (OR)	95% Confidence Intervals (CI)	P-value
Eating outside last week:					
No	35/62 (56.5%)	103/124 (83.1%)	Ref.		
In the pub	**10/45 (22.2%)**	**8/111 (7.2%)**	**3.7**	**1.3-10.1**	**0.008**
In the street	**17/52 (32.7%)**	**13/116(11.2%)**	**3.9**	**1.7-8.7**	**0.001**
Eating outside	**27/62 (43.5%)**	**21/124 (16.9%)**	**3.8**	**1.9-7.5**	**<0.001**
Foods eaten last week:					
Shellfish/seafood	13/62 (21%)	27/124 (21.8%)	Ref.		
Ice-cream manufactured by	4/17 (23.5%)	6/33 (18.2%)	1.4	0.3-5.8	0.654
Home made ice-cream	6/19 (31.6%)	6/33 (18.2%)	2.1	0.6-7.7	0.270
Fresh milk	39/52 (75%)	47/74 (63.5%)	1.7	0.8-3.8	0.173
Local beer	9/22 (40.9%)	7/34 (20.6%)	2.7	0.8-8.8	0.100
Salad/fruits	**17/30 (56.7%)**	**13/40 (32.5%)**	**2.7**	**1.0-7.2**	**0.043**
Half-cooked beef	**14/27 (51.9%)**	**6/33 (18.2%)**	**4.9**	**1.5-15.5**	**0.006**
Roasted meat	**22/35 (62.9%)**	**7/34 (20.6%)**	**6.5**	**2.2-19.1**	**<0.001**
Source of water:					
Municipally supplying water	20/62 (32.3%)	46/124 (37.1%)	Ref.		
Rainwater	9/29 (31%)	12/58 (20.7%)	1.7	0.6-4.7	0.288
Well	41/61 (67.2%)	78/124 (62.9%)	1.2	0.6-2.3	0.565
Pond	**11/31 (35.5%)**	**6/52 (11.5%)**	**4.2**	**1.4-13.0**	**0.009**
Washing hand before eating:					
Usually	13/62 (21%)	26/124 (21%)	Ref.		
Sometime	40/53 (75.5%)	83/109 (76.1%)	0.96	0.5-2.1	0.925
Never	9/22 (40.9%)	15/41 (36.6%)	1.2	0.4-3.5	0.736
Washing hand after go to toilet:					
Usually	31/62 (50%)	47/124 (37.9%)	Ref.		
Sometime	22/53 (41.5%)	66/113 (58.4%)	0.5	0.3-1.0	0.042
Never	9/40 (22.5%)	11/58 (19%)	1.2	0.5-3.3	0.670
Take a bath:					
Private bathroom	52/62 (83.9%)	115/124 (92.7%)	Ref.		
Sharing bathroom	9/61 (14.8%)	8/123 (6.5%)	2.5	0.9-6.8	0.069
River/pond	1/53 (1.9%)	1/124 (0.9%)	2.2	0.1-36.1	0.568

Table 14 (cont.): Prevalence of risk factors associated with DEC disease in univariate analysis (II)

Potential risk factors	DEC (Cases = 62)	Diarrhoea non DEC (Controls = 124)	Matched Odd Ratio (OR)	95% Confidence Intervals (CI)	P-value
Keeping food:					
In refrigerator	20/62 (32.3%)	46/124 (37.1%)	Ref.		
In food tray with a cover	19/39 (48.7%)	41/87 (47.1%)	1.1	0.5-2.3	0.869
In the kitchen without a cover	23/43 (53.5%)	37/83 (44.6%)	1.4	0.7-3.0	0.342
Type of latrines:					
Dry latrine:	12/62 (19.4%)	23/124 (18.5%)	Ref.		
Modern toilet:	36/48 (75%)	82/105 (78.1%)	0.8	0.4-1.9	0.672
Wet latrine :	7/19 (36.8%)	10/33 (30.3%)	1.3	0.4-4.4	0.628
Excrete directly into the field/fishpond:	7/19 (36.8%)	9/32 (31.4%)	1.5	0.5-5.0	0.517
Household garbage:					
Rubbish pit	52/62 (83.9%)	116/124 (93.5%)	Ref.		
Open surrounding	**10/62 (16.1%)**	**8/124 (6.5%)**	**2.8**	**1.0-7.5**	**0.035**
Dispose of waste water:					
Sewage system	47/62 (75.8%)	109/124 (87.9%)	Ref.		
Pond	7/54 (13%)	10/119 (8.4%)	1.6	0.6-4.5	0.351
Garden	**8/55 (14.5%)**	**5/114 (4.4%)**	**3.7**	**1.2-11.9**	**0.02**
Kinds of livestock:					
Dogs	25/62 (40.3%)	42/124 (33.9%)	Ref.		
Cats	3/28 (10.7%)	8/50 (16%)	0.6	0.2-2.6	0.52
Chickens	19/44 (43.2%)	21/63 (33.3%)	1.5	0.7-3.4	0.30
Pigs	23/48 (47.9%)	22/64 (34.4%)	1.8	0.8-3.8	0.148
Cattle	9/34 (26.5%)	5/47 (10.6%)	3.0	0.9-10.0	0.063
People in the house:					
Under 5	53/62 (85.5%)	95/124 (76.6%)	Ref		
Over 6	9/62 (14.5%)	29/124 (23.4%)	0.6	0.3-1.3	0.157
Income:					
High	10/62 (16.1%)	29/124 (23.4%)	Ref.		
Average	36/46 (78.3%)	67/96 (69.8%)	1.6	0.7-3.6	0.290
Low	16/26 (61.5%)	28/57 (49.1%)	1.7	06-4.3	0.293

The factor **no contact with diarrhoea patient** in categorization of **contact with diarrhoea patient** was used as a reference for the comparisons among different factors. The factor **having contact with diarrhoea patient** (OR: 2.4, 95%CI: 1.1-5.4, P: 0.027) was significantly associated with DEC disease.

The factor **civil servant/teacher/food industry/health-worker/student** in categorization of **occupation** was used as a reference for the comparisons among different factors. The factor **food vendor** (OR: 4.2, 95%CI: 1.3-13.6, P: 0.013) was strongly associated with DEC disease.

The factor **never eating outside** in categorization of **often eating outside** was used as a reference for the comparison among different factors. The factors **eating out side 1-2 times/month** (OR: 4.7, 95%CI: 1.5-12.5, P: 0.005) and **eating outside 1-2 times/week** (OR: 3.9, 95%CI: 1.3-11.9, P: 0.011) were strongly associated with the disease. When these factors were combined into one factor called **eating outside more than 1-2 times/month,** it was also significantly associated with the DEC disease (OR = 4.2, 95%CI 1.8 – 9.6, P = <0.001).

The factor **no eating outside last week** in categorization of **where to eat outside last week** was used as a reference for the comparisons among different factors. The factors **eating outside last week in the pub/restaurant** (OR: 3.7, 95%CI: 1.3-10.1, P: 0.008) and **eating outside last week in the street** (OR: 3.9, 95%CI: 1.7-8.7, P: 0.001) were significantly associated with DEC disease. When these factors were combined to be the factor **eating outside last week**, it was also found to be significantly associated with the DEC disease (OR = 3.8, 95%CI 1.9 – 7.5, P = <0.001).

The factor **shellfish/seafood** in categorization of **foods eating last week** was used as a reference for the comparisons among different food factors. The factors **salad/fruits** (OR: 2.7, 95%CI: 1.0-7.2, P: 0.043), **half-cooked beef** (OR: 4.9, 95%CI: 1.5-15.5, P: 0.006) and **roasted meat** (OR: 6.5, 95%CI: 2.2-19.1, P: <0.001) were strongly associated with DEC disease.

The factor **municipally water supply** in categorization of **source of water for domestic use** was used as a reference for the comparison among different water factors. The factor **pond water** (OR: 4.2, 95%CI: 1.4-13.0, P: 0.009) was strongly associated with DEC disease.

The factor **rubbish pit** in categorization of household garbage was used as a reference for the comparison among different factors. The factor **open surrounding** (OR: 2.8, 95%CI: 1.0-7.5, P: 0.035) was strongly associated with DEC disease.

The factor **sewage system** in categorization of **dispose of waste water** was used as a reference for the comparisons among different factors. The factor **garden** (OR: 3.7, 95%CI: 1.2-11.9, P: 0.020) was strongly associated with the DEC disease.

Multivariate analysis:

The risk factors that were significantly associated with diarrhoeal disease in the univariate analysis were chosen for multivariate analysis.

Variables considered for the model include the following:

- Have contact with diarrhoeal patients.
- Food vendor
- Eating outside more than 1-2 times/month
- Eating outside last week
- Eating salad/fruits last week:
- Eating half-cooked beef last week:
- Eating roasted meat last week:
- Pond water
- Garbage in open surrounding
- Dispose of waste water to garden

Table 15: Result of the multivariate analysis (III)

Potential risk factors	Matched Odd Ratio (OR)	95% Confidence Intervals (CI)	P-value
Having contact with diarrhoeal patient	1.7	0.6-4.7	0.3
Food vendor	3.3	0.8-13.6	0.1
Eating outside more than 1-2 times/month	**3.2**	**1.1-9.1**	**0.03**
Eating outside last week	1.6	0.6-3.8	0.3
Salad/fruits eating last week	1.1	0.3-3.6	0.9
Half-cooked beef eating last week	*3.5*	*~1.0-12.4*	*0.056*
Roasted meat eating last week	**7.4**	**2.2-24.3**	**0.001**
Pond water	**4.7**	**1.4-16.3**	**0.014**
Open surrounding garbage	1.7	0.5-5.7	0.4
Dispose of waste water *to Garden*	2.8	0.7-12.1	0.2

In the multivariate analysis for DEC & Diarrhoea Non-DEC groups, conditional logistic regression was used. Three of 10 exposures consisting of **eating outside more than 1-2 times/month, roasted meat eating last week, pond water** increased the risk of illness as shown in table 15. Other exposures, though they were risk factors in the univariate analysis, turned out to be independently associated with diarrhoeal disease when being included in the model of conditional logistic regression

Chapter V: Discussion

The risk factors associated with diarrhoeal disease and DEC disease may be different between endemic areas. The question is ‘what are local risk factors for diarrhoeal disease and DEC disease?’ In an endemic area, people are exposed the pathogens through multiple vehicles. The question becomes ‘why do some people develop a disease and the others do not?’

In this study, we matched cases and controls by age and sex in order to identify the risk factors for diarrhoeal disease and DEC disease.

5.1. Strength of the study

This study was conducted in the hospital, so it was easy to select the diarrhoeal patients, non-diarrhoeal patients and collect stool samples for the study. And we can interview cases and controls at the same time.

The diagnosis of DEC or other enteric pathogens were conducted at Enteric Pathogens Laboratory-NIHE-Viet Nam and with a highly qualified staff and high quality materials and equipments with molecular supported from University of Ryukyu, Japan. In these conditions, the diagnoses of DEC cases are highly reliable.

The Duc Giang Hospital was selected for study because it was not far from the laboratory at NIHE and stools specimens were easily transported to the laboratory. In addition, it was easy for different research teams to meet and exchange experiences and solve technical problems.

Interviewers used Vietnamese. Fortunately, all the cases proved to be ethnically Kinh whose mother tongue is Vietnamese.

The questionnaires had been translated by a special translator who has good command of English and of community health to assure the validity and reliability of the instrument used in the study. Pre-testing proved that questions were correctly understood.

Before the data collection process started, the research team was trained in interviewing and of laboratory techniques to ensure good quality work. Staff seminars were conducted to report progress, discuss problems, and maintain good moral during the data collection. After interviews, the interviewers cross-checked the questionnaires to identify missing answers and went back to the respondents to complete questionnaires if necessary.

The necessary budgets for the study were supported from NORAD; Department for General Practice and Community Medicine, Oslo; NIHE, Ha Noi and Ryukyu University, Japan.

The close collaboration with the hospital staff made it easy to maintain a high motivation and will greeting facilitate feedback of the final result and the implementation of any recommendations.

5.2. Weaknesses of the study

This study was conducted in the hospital, so, we can not account for prevalence of the disease in the community.

Time for conducting the study was limited. The field study was conducted in six months only. In northern Viet Nam, the rainy season (wet season) starts in May and stops at the end of August. The wet season is also the main season for enteric diseases cause by entero-bacteria. The study included only the last month of the wet season. Therefore, we could not collect as many cases as we expected. Furthermore, results of the study only exhibit the distribution of cases during 6 months and we could not describe the seasonal distribution of cases.

Some risk factors were difficult to quantify. For example, it was difficult to define the economic level of the study subjects. Sometimes, they did not know their income exactly. Household goods and number of domestic animals could only serve as proxies for the economic level.

Most diarrhoeal patients stay in the hospital only a few days for treatment. Since it took several days to diagnose a DEC case, we could not wait for the results of laboratory to choose controls for interviews. Accordingly, we had to interview patients (including both cases and controls) before they left for home. Then cases and controls were selected depending on the results of laboratory together with findings from interviews. However, this difficultly indirectly secured that the study staff was effectively blinded during the data collection.

Due to cost and work load time to diagnose DEC cases by PCR method, we could not do this test every day. We sometimes kept suspected DEC strains until the next PCR run. Normally we did PCR twice a week.

The limited budget prevented us from drug sensitivity testing of the *E. coli* strains.

5.3. Characteristic of the sample.

Geographical distribution:

In this study, 43 cases (69.35%) were found in suburban Gia Lam District, 19 cases (36.35%) were found in urban Long Bien District.

This result showed that DEC-related diarrhoea is associated with densely populated areas where most residents are rice growing and livestock and poultry raising farmers, e.g. Gia Lam. This district is also one of the poorest areas in Ha Noi.

Temporal distribution:

18 sporadic cases were reported in August 2005, which was higher than other months in the study. This was summer time and there were many activities for children. Also, it rained a lot during this time. Thus, findings of the study showed similarity with other studies observing an increasing number of diarrhoea cases occurring during summer time and related to rainfall.

In November, the temperature in the province was still high for the season and it was still raining. Surface water might have been contaminated with faeces of people. Wells could have been overflowed and contaminated with surface water.

We found no clear outbreaks in this area of Long Bien and Gia Lam Districts.

Age and sex distribution:

Most of the cases were among children under 5 years of age (79%). Not many DEC cases were found in the adult group. This finding was similar to some other studies (9;10). Perhaps, adults have acquired immunity against DEC disease. Or they may buy antibiotics to treat the disease by themselves. In Viet Nam, it is easy to buy medicine in the pharmacy without a doctor's prescription. We found that adult diarrhoea patients often used antibiotic before going to the hospital; hence this may have influenced on the diagnosis of DEC.

In this study, 52.2% and 46.8% of the cases were males and females respectively. There was no significant difference between males and females in sex distribution. This result proves that as most of the study cases are aged around 5 years, there is no connection between occupations and

infection. Likewise, the population is concentrated in a geographical area, thus there is no association between customs and gender-based infection.

Laboratory results:

In this study, 48.8% of the cases were EAEC. It is similar to a study conducted in Viet Nam (2). This result shows that EAEC strains are most dominant as the cause of *E. coli*-inflicted diarrhoea in children in Viet Nam presently. These strains are also associated with persistent diarrhoea in children, a disease which causes many Vietnamese doctors difficulties while making diagnoses and determining its treatment. The Multiplex PCR technique used for diagnosing DEC is at the moment hardly available in hospitals.

EHEC/STEC strain was not found in this study, yet some other studies conducted Viet Nam identified its presence in human and pig's faeces (104),(112).

5.4. Risk factors for diarrhoeal disease:

When data from the two groups of diarrhoeal disease (186 cases) were compared to non-diarrhoeal disease (62 controls), two factors were found significantly associated with diarrhoeal disease including: **Negligence washing hands before eating** and **keeping food outside the fridge.**

64.5% of diarrhoea patients reported that their families often kept food outside the fridge while there were only 33.9% of Non-diarrhoea patients whose families did this. The families that kept the food outside the fridge were often families with low economic status. They may not have enough money to buy a refrigerator or they may not know that keeping food in the fridge is important for avoiding foods contamination. These families often live under poor hygienic conditions, in poor housing, and without safe water supply. In the rural areas of Viet Nam, especially in rice fields, flies and other vectors of diarrhoea disease transmission are found in every kitchen. These vectors might transport bacteria to food, food trays, and other household articles. In addition, the temperature is high in the summer and facilitates the growth of bacteria.

Moreover, most of families in the study area used well water (119/124 in diarrhoea group, 37/62 in non-diarrhoea group) or pond water as a main source of water for domestic use like preparing food, washing-up, having shower, washing clothes, etc. Well water and pond water may be contaminated by human and animals faeces.

Both the high density of flies and the usage of untreated water might explain the significant association between keeping food outside the fridge and diarrhoeal disease.

There were 79% diarrhoea patients and 29% non-diarrhoea patients who said that they rarely wash their hands before eating**.** Most patients in the study were children (289/375). Children are often eating by hand. This finding is supported by other (87).

The two risk factors identified, however, are just traits of a great context: poverty, lack of safe water and sewage and poor hygiene practices.

5.5. Risk factors for DEC disease: (The comparison between groups of DEC disease and non-diarrhoeal disease).

Three factors were found significantly associated with DEC disease: **Having contact with diarrhoea patients*;* negligence washing hands before eating** and **keeping food outside the fridge**.

The factors **negligence washing hand before eating** and **keeping food outside the fridge** were significantly associated with DEC disease. These results were as same as results of analysis between groups of Diarrhoea patients and Non-diarrhoea patients.

There were 24.2% of DEC patients and 8.1% of Non-diarrhoea patients said that they did **have contact with diarrhoea patients**. It is a common habit of people in the study area to visit a sick person before or after that person coming to or returning from the hospital. While visiting the diarrhoea patient house, friends and neighbours may be infected by DEC through drinking tea or eating some foods.

The study showed that diarrhoeal diseases in most cases were caused by EAEC (30/62), a group of DEC related to persistent diarrhoeal disease. When persistent diarrhoea is not completely treated, it may be a risk factor for person-to-person transmission.

5.6. Risk factors for DEC disease: (The comparison between groups of DEC disease and diarrhoea non-DEC disease).

Three factors were found significantly associated with DEC disease: **pond water** in categorization of **source of water for domestic use*;* more than 1-2 times/month** in categorization of **often eating outside** and **roasted meat** in categorization of **foods eaten last week**.

Pond water:

There were 17.7% of diarrhoea patients using **pond water** for domestic purpose, but the percentage of diarrhoea non-DEC patients using **pond water** for domestic purpose was only 4.8%.

In Viet Nam, especially in rural areas, it is not easy for every family to get safe water for domestic use. It depends on geographical areas, infrastructure supply, and the supply capacity of water plant. In Long Bien and Gia Lam districts, people in some areas still do not have access to sufficient pipe water. During summer, when the water consumption rises dramatically, some families face shortage of hygiene water. They may have to find different ways to store water, or have to find additional sources of water for domestic use. One of such additional water sources is pond water which is contaminated by faeces of human and livestock. This explains the fact that people using pond water for domestic use stand a higher risk of DEC-related diseases than those in other groups.

Eating outside more than 1-2 times/month:

There was 22% DEC patients said that they often eat outside more than 1-2 times/month while only 8.9% of other diarrhoea patients reported to do so. This result might refer that food both on street and in pubs and restaurants in this area are under hygiene conditions.

Eating outside in Viet Nam nowadays become more common. The reason for people to eat outside could be related to works, habit, custom, enjoying, taste, etc. The places for eating outside as restaurants, pubs, markets, etc are always available 24h a day. However, Ha Noi health service is not able to manage the quality of the street food, especially in rural areas.

Most people in Vietnam have average or low income and so their choices of restaurants are usually cheap places. However, cheap food is naturally goes with low quality. This fact is particularly true in

rural areas and it also explains the higher rate of DEC patients among those who eat out at least 1-2 times per month compared to that in other diarrhoea patient group

Roasted meat:

There was a 35.48% DEC patients eating **roasted meat** last week**,** there was only 11.29% in diarrhoea non DEC patients. In some study, **roasted meat, half-cooked beef** were related with DEC disease(5).

Roasted meat is a popular dish in Vietnamese families due to its tastiness and simple cooking method. After being cleaned and thinly sliced, pork meat is seasoned with more spice then grilled. A most common way in Vietnam is using coal to grill meat, thus the heat is not so evenly allocated on the surface of each piece of meat. Hence, it is very likely that certain parts of the meat are not well done and that the bacteria are not killed by high temperature. Consequently, any person who eats this kind of meat risks diarrhoea.

Furthermore, a study (104) conducted in Vietnam showed that the prevalence of DEC is extremely high among livestock, especially pigs. This is why eating roasted meat increases the risk of DEC-induced diarrhoea.

Chapter VI: Conclusions and Recommendations

6.1. Conclusions:

- Diarrhoeal disease caused by DEC in Duc Giang Hospital is mostly associated with EAEC strains. Cases of diarrhoea caused by EHEC, *Vibrio cholara, Salmonella* were not found.

- Diarrhoea is very much associated with use of unsafe water, eating without hand-washing and inadequate food storing.

- Diarrhoeal disease caused by DEC is closely linked to contacts with diarrhoeal patients, eating roasted meat and food sold by street vendors.

6.2. Recommendations: (for prevention of both diarrhoeal disease and DEC disease)

- **To the policy makers**
  - **Safe water should be provided to the communities**
  - **Regular quality control of foods and beverages sold in restaurants**

- **Hygienic practices should be promoted by providing community health education on the importance of**
  - **Washing hands before eating**
  - **Boiling drinking water**
  - **Storing food in refrigerators (for those who have it),**
  - **Warming up food before eating**
  - **Hand and food hygiene after contact with a patient who has diarrhea**

The following studies can be conducted in order to increase the effectiveness of diagnosis and treatment of diseases caused by DEC, particularly persistent diarrhoea originated from EAEC: "Study on the characteristics of DEC strains and their anti-biotic resistant status"

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Annex 1: Information sheet for Consent to Participate in Research (Exit Interviews)

Patient's Name: _____ Study Identification Number: _____

Dear Madam/ Sir,

The Department of Enteric Pathogens in National Institute of Hygiene and Epidemiology (NIHE)-Ha Noi in collaboration with the Institute of International Health in Oslo University, Norway are carrying out a study among diarrhoea patients in Duc Giang Hospital, Ha Noi, Viet Nam

Diarrheagenic *E. coli* (DEC) diseases are common among infant and children. The study will be conducted with the interrelated objectives to find out how frequent DEC is the cause of diarrhoea in patients in Duc Giang Hospital and the risk factors associated with transmission DEC disease in this area.

The questions will cover information on social demographic data, family economic level and possible sources of infection.

I have few questions about diarrhoea and related issues. Your answers will be written and then used for analysis. All information you provide will be handled as confidential and your individual answers will not be known, excepting the interviewer and the coordinator of this study. The results will be used only to improve strategies for prevention of diarrhoea, one of the most common diseases in the community.

We will need at least 20 minutes to discuss and record the information. You can withdraw from the interview at any stage if you do not wish to continue.

Will you participate in this study? Yes ☐ No ☐

Do you have any question?

I (the interviewer) hereby confirm that the patient has given his consent to participate in the study.

Interviewer signature: ----------	Researcher: **Nguyen Dong Tu** MPhil in International Community Health 2004-2006 Oslo, University.

Annex 2:

Questionnaire for identification of the risk factors associated with transmission diarrheagenic *E. coli* disease in Duc Giang district, Ha Noi, Viet Nam.

Confidential interview schedule for patients

Status of the patients:

1. Case (Positive with pathogenic *E. coli* gene)
2. Control 1 (others diarrhoea)
3. Control 2 (non-diarrhoea)

(For children under 5, we will ask mothers/guardians for the information)

Date of interview: ____/_____/____

Questionnaire situation:

Complete : ☐

Incomplete : ☐

Reasons if is incomplete: _____

Study serial no: _____

I. PERSONAL DEMOGRAPHIC INFORMATION:

1. Study Identification Number:

2. Address:

3. Ages: Date of birth: ____/____/____ (dd/mm/yy)

4. Sex: ☐ Male ☐ Female

5. Education: ☐ 1. Illiteracy
2. Primary
3. Junior secondary
4. Senior secondary
5. Bachelor.

6. Marital status: ☐ 1. Single 4. Widowed
2. Married 5. Separated
3. Divorced

7. Ethnic: ☐ 1. Kinh 3. Nung
2. Tay 4. Other:……

II. POSSIBLE SOURCES OF INFECTION (for both case and control):

1. Have you contact (work colleagues, school mates, relatives, neighbours….) with diarrhoeal patients within one month before? 1. Yes
2. No
3. Don't know

If yes, where? 1. In household 4. In hospital
2. Neighbouring 5. In the school
3 Working place

2. Occupations: 1. Farmer/ fisher 6. Unemployment
2. Breeder 7. Student , precise the level
3. Civil servant, teacher, office clerk 8. Food vendor
4. Food industry worker 9. others:
5. Health worker ……

Working related with food activities:

3. Did you travel outside your community recently (last month)? 1. Yes
2. No

If yes, Where?

4. Do you often eat outside home? 1. rarely 3. 1-2 times/week
2. 1-2 times/month 4. Never

5. Where did you eat during last week before disease? 1. In the restaurant/pub 3. No
2. In the streets

6. What did you eat these types of food last week?

Types of food		Where?
Shellfish/seafood	☐	
Raw fish	☐	
Half-cooked beef	☐	
Roasted meat	☐	
Fresh milk	☐	
Local beer	☐	
Salad/fruits	☐	
Ice-cream home made	☐	
Ice cream small scale industry	☐	

7. Source of water for domestic use? 1. Municipal water supply 3. Pond
2. Wells 4. Rainfall water

8. Do you wash your hands before eating? 1. Never 2. Sometimes 3. Usually

9. Do you wash your hands after going to toilet? 1. Never 2. Sometimes 3. Usually

10. Where do you take your bath? 1. River 3. Bathroom share with other family
2. Private bathroom

11. How do you keep your food in your family? 1. In refrigerator
2. In the kitchen without a cover
3. In food tray with a cover

12. Type of latrines
1. Modern toilet
2. Wet latrine
3. Dry latrine
4. Excrete direct into fishponds or environmental

13. Where do you dispose of household garbage? ☐
1. Rubbish pit
2. Open surrounding
3. Other: ……..

14. Where do you dispose of waste water? ☐
1. Sewage system
2. Pond; 3. Garden
4. Other: …..…

15. Do you have following kinds of livestock? If yes, state size of herd.
1. Cattle _____ 4. Pig _____
2. Goats _____ 5. Chickens _____
3. Dog _____ 6. Other _____ _____

III. FAMILY ECONOMIC LEVEL INFORMATION

1. How many people are living in this house? ☐☐

2. What kind of items do you have in your family?
1. Car 5. Television
2 Motor cycles 6. VCD or DVD
3. Bicycle 7. Others.
4. Radio ……

3. How much money does your family earn every month? -------, --------, ------ VND

4. Material of the house builds by
1. Brick 3. Wood
2. Concrete 4. Bamboo

5. How many rooms in your house? ---- ----

6. Do you have electricity at home? 1. Yes. 2. No.

IV. CLINICAL DATA (for case & control 1)

1. Hospitalized on: ……/……. / 2005

2. Weight: ……kg. Height: ……cm.

3. Temperature: …….. ^{0} C.

4. Number of days with diarrhea: ..…… days.

5. Stool frequency per day: ……

6. Is there blood in stool? Yes ☐ No ☐ Don't know ☐

7. Have you vomited? Yes ☐ No ☐

If yes, state vomiting frequency per day: ☐

8. Patient's dehydration status at admission? ☐	1. None 2. Mild	3. Moderate 4. Severe
9. Impression from the interviewer about the personal hygiene of the subject (observation)	1. Very clean 2. Clean	3. Dirty 4. Very dirty

9. Treatment before hospitalisation: .....

.....

.....

10. Treatment during hospitalisation: .....

.....

.....

11. Stool sample collected on: ……/……/ 2005.

12. Date of discharge: _____

13. Is there any complication? Yes.☐ No.☐

If yes describe: .....

.....

.....

FOR CONTROL 2 ONLY: (Patients non-diarrhoea)

Have you had diarrhoea during the last 7 days? Yes.☐ No.☐

If yes, what is diarrhoea disease did you get? _____

V. LABORATORY INFORMATION

Date of sample collection: ……/……/……

Results exam:

1	Positive	☐
2	Negative	☐

If positive, what type of gene does it has?

	Gene	
1	AggR	☐
2	eae	☐
3	elt	☐
4	est	☐
5	ipaH	☐
6	Stx	☐

DATE: ___/___/___.

Interviewer name_____

Annex 3: Questionnaires formulation

Factor	Variable	Indicator	Scale of measurement
I. Personal demographic information	Age	Age at last birthday	Day / Month / Year Age group: 1 = 0 – 5 years 2 = 6 – 10 years 3 = 11 – 15 years 4 = 16 – 20 years 5 = 21 – 25 years 6 = 26 – 30 years 7 = ≥ 31 years
	Gender	Basic observation	Male / Female
	Marital status	Response to a specific question	Single/Married/Divorced/Widowed/Separated
	Ethic	Homogenous / Indigenous	
	Religion		
	Education	The highest grade of year or regular	1 = Illiteracy 2 = Primary school 3 = Junior Secondary 4 = Senior Secondary 5 = Bachelor
II. Risk factor associated with DEC disease.	Contact with diarrhoeal patients		Yes/no/don't know
	Occupation	Breeder Civil servant, teacher, office clerk Food Industry worker Farmer, fisher Health worker Food vendor Unemployment Student, precise the level	1 = Farmer, fisher 2 = Breeder 3 = Civil servant, teacher, office clerk 4 = Food Industry worker 5 = Health worker 6 = Unemployment 7 = Student, precise the level 8 = Food vendor

		Other precise	9 = Other precise
	Travel outside the community recently		Yes/no
	Eating outside		
		Rarely	1 = rarely
		1-2t/month	2 = 1-2t/month
		1-2t/week	3 = 1-2t/week
		Never	4 = nerver
	Where to eat outside		
		In the restaurant/pub	1 = In the restaurant/pub
		In the streets	2 = In the streets
		No	3 = No
	Types of food ate last week	Shellfish/seafood	1 = Shellfish
		Raw fish	2 = Raw fish
		Half-cooked beef	3 = Raw beef
		Roasted meat	4 = Meat
		Fresh milk	5 = Fresh milk
		Local beer	6 = Local beer
		Salad/fruits	7 = Salad
		Ice-cream home made	8 = Ice-cream home made
		Ice cream small scale industry	9 = Ice cream small scale industry
	Where to keep the foods	In refrigerator	1 = In refrigerator
		Kitchen	2 = In the kitchen without a cover
		Grade manger	3 = In food tray with a cover
	Sources of water for domestic use.	Municipal water supply	1 = Municipal water supply
		Wells	2 = Wells
		Pond	3 = Pond
		Rainfall water	4 = Rainfall water
	Washing hands before eating	Never	1 = Never
		Sometime	2 = Sometime

		usually	3 = usually
	Washing hands after go to toilet	Never	1 = Never
		Sometime	2 = Sometime
		usually	3 = usually
	Where to have bath	River	1 = River
		Private bathroom	2 = Private bathroom
		Share bathroom with others family	3 = Share bathroom with others family
	Type of latrine		
		Model toilet	1 = Model toilet
		Wet Latrine	2 = Wet Latrine
		Dry Latrine	3 = Dry Latrine
		Excrete direct in to fish-pond or environmental	4 = Excrete direct in to fish-pond or environmental
	Place of disposal household garbage	Rubbish pit	1 = Rubbish pit
		Open surrounding	2 = Open surrounding
		Other: ……..	3 = other
	Place of disposal household water	Sewage system	1 = Sewage system
		Pond	2 = Pond
		Garden	3 = Garden
		Other: …..…	4 = others
	Kinds of livestock	Cattle	1 = Cattle
		Goats	2 = Goats
		Dog	3 = Dog
		Pig	4 = Pig
		Chickens	5 = Chickens
		Other _____	6 = Others

III. Family economic level	Number of people in household		1 = 1 / 4 persons 2 = 5 – 6 persons 3 = > 7 persons
	Kind of items in family	Car Motor cycles Bicycle Radio Television VCD or DVD Others.	1 = Car 2 = Motor cycles 3 = Bicycle 4 = Radio 5 = Television 6 = VCD or DVD 7 = Others
	Income	< 30 USD/month/person 30-60 USD/month/person > 60 USD/month/person	1 = < 30 USD/month/person 2 = 30-60 USD/month/person 3 = > 60 USD/month/person
	Material of the house	Brick Concrete Wood Bamboo	1 = Brick 2 = Concrete 3 = Wood 4 = Bamboo
IV. Clinical data	Clinical symptoms	Fever Headache Vomit Diarrhoea	Yes/No Yes/No Yes/No Yes/No
	Treatment before hospitalisation		Dependent on answer/response
	Treatment during hospitalisation		Dependent on answer/response
	Lab. information		
Date interview Name of interviewer			

ANNEX 4: GANTT CHART

	Tasks to be performed	2005										2006					
		Mar.	Apr.	May.	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May.	Jun.
1	Prepare proposal																
2	Finalising proposal																
3	Clearance orientation of local gov. and institution																
4	Pretest proposal																
5	Training interviewers																
6	Training in lab.																
7	Field testing questionnaires																
8	Collecting data																
9	Discussion and recommendation																
10	Monitoring research project																
11	Data analysis																
12	Report finalisation																
13	Reporting																
14	Thesis defence																

Annex 5: Table 1: Classified diarrhoeagenic *E. coli* (113)

	Abbrev.	**Definition**	**Type of disease**
Verocytotoxin (Shiga toxin)-producing *E. coli*	VTEC or STEC/ EHEC	*E. coli* that produce verocytotoxin (Shiga toxin) VT1 and/or VT2	Diarrhoea, haemorrhagiccolitis, haemolytic uraemicsyndrome (HUS)
Enterotoxigenic *E.coli*	ETEC	*E. coli* that produce enterotoxins that are heat stable (STh, STp) and/or heat labile (LT)	Acute watery diarrhoea
Attaching and effacing *E. coli*	A/EEC	*E. coli* that attach to and efface the microvilli of enterocytes, but do not produce high levels of verocytotoxin	Acute or persistent diarrhoea
Enteropathogenic *E. coli*	EPEC	Subtype of A/EEC, usually of particular serotypes that mostly contain an EPEC adherence factor plasmid and often produce bundle-forming pilus (BFP)	Acute or persistent diarrhoea
Enteroaggregative *E. coli*	EAEC	*E. coli* that exhibit a pattern of aggregative adherence to tissue culture	Acute watery, often Protracted diarrhoea
Diffuse adherent *E. coli*	DAEC	*E. coli* that exhibit a pattern of diffuse adherence to tissue culture	Acute or persistent diarrhoea
Enteroinvasive *E. coli*	EIEC	*E. coli* that share virulence determinants with *Shigella* spp.	Acute, often inflammatory diarrhoea; dysentery