Analysis of Microbial Pollution in Shallow Groundwater and Its Health Impact at Household Level in a Developing Country

（開発途上国における浅層地下水の微生物汚染と世帯レベルの健康影響の解析）

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Abstract

Insufficiency of piped water supply is a major concern in developing countries especially in the urban centres including the Kathmandu Valley of Nepal, where population is growing rapidly. Because of easy and inexpensive installation and maintenance of dug and tube wells, shallow groundwater is being used as private self-supply by households but it is often excluded from official statistics and taken as granted by government as well as society. Groundwater is serving as drinking water source but mainly used for bathing, washing and other domestic purposes. Microbial contamination of groundwater has been reported in many developing countries where diarrhoeal occurrence has been a simultaneous and major health problem. However, researches that aid to develop effective groundwater pollution control strategies such as examining spatial and seasonal variation of microbial quality are lacking. Although assessment of public health safety of any kind of water sources has been advised by World Health Organization, discussion remains in quantitative estimation of health risk from using contaminated groundwater. In addition, association of groundwater microbial quality with diarrhoeal occurrence at the household level was still unexplored.

This study aimed; a) to examine seasonal variations of microbial quality in shallow groundwater and explore possible mechanisms of the variation (Chapter 3); b) to estimate risk of diarrhoea from prevailing enteropathogenic microorganims in groundwater (Chapter 4); c) to examine the association between groundwater microbial quality and diarrhoea occurrence in households while controlling other potential risk factors (Chapter 5). In this study, dug and tube wells were assessed for *Escherichia coli* (*E. coli*) and total coliforms during dry and wet seasons from 2009 to 2012. A few wells in wet season of 2009 and 2010 were assessed for presence of enteropathogenic microorganims; *Giardia* cysts and *Cryptosporidium* oocysts.

Chapter 3 showed that, microbial concentrations in shallow groundwater were significantly higher during wet season than during dry season, especially in dug wells. Analyses of rainfall and *E. coli* concentrations in different seasons indicated that a high level of faecal material infiltrating during rainy season might have caused the seasonal variations. A moderate to strong relationship between *E. coli* concentrations and water level below ground surface suggested that the rise in groundwater levels during wet season might be another reason for this variation.

Chapter 4 showed that risk of diarrhoea from Enteropathogenic *E. coli* (EPEC) in dug wells and from *Cryptosporidium* and *Giardia* (protozoa) in both dug and tube wells were higher than the acceptable limit ($<10^{-4}$ infections/person-year) for both drinking and bathing exposures. Risk from protozoan enteropathogenic microorganisms increased the total risk 10,000 times, indicating that ignoring protozoa could lead to serious underestimation of risk from water sources. Bathing exposure considerably increased risk, indicating that it is important pathway. Point-of-use (POU) water treatment method decreased the risk six fold and had the largest impact on total risk.

A method of integrating techniques of Geographic Information System (GIS) with epidemiological data was developed in Chapter 5, which could be helpful in data poor regions. Households using groundwater had higher tendency (p-value > 0.05) to report diarrhoea and those using highly contaminated groundwater for bathing purpose were at a greater risk of diarrhoea occurrence (adjusted odds ratio (AOR) = 5.21, p-value < 0.05).

These results underscored the microbial quality in groundwater as an important factor of health risk for diarrhoea occurrence at household level in the valley. In addition, this study highlighted the importance of bathing exposure pathway and suggested small-scale intervention study for developing diarrhoea risk reduction strategies. Our findings are specifically representative of the urban areas of developing countries which are depending on shallow groundwater to mitigate water problem and which have socio-economic and cultural similarities with our study area.

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Abbreviation and Notation

ADB	Asian Development Bank
ADWO	Asian Drinking Water Outlook
APWF	Asia-Pacific Water Forum
BRB	Bagmati river basin
CBS	Central Bureau of Statistics
CREEW	Center of Research on Energy Environment and Water
CWF	Ceramic Water Filter
DHM	Department of Hydrology and Meteorology
ESRI	Environmental System Research Institute
GEE	Generalized Estimation Equation
GIS	Geographic Information System
HMG/N	His Majesty’s Government of Nepal
JICA	Japan International Cooperation Agency
KMC	Kathmandu Metropolitan City
KUKL	Kathmandu Upatyaka Khanepani Limited
LSMC	Lalitpur Sub-Metropolitan city
MCS	Monte Carlo Simulation
MDG	Millennium Development Goal
MLD	Million liters per day
MPN	Most probable number
MoHP	Ministry of Health and Population
POU	Point of use
QMRA	Quantitative microbial risk assessment
UN	United Nations
UNEP	United Nations Environment Programme
UNICEF	United Nations Children’s Fund
USEPA	United States Environmental Protection Agency
VDC	Village Development Committee
WHO	World Health Organization

Table of contents

Chapter 1
Introduction

Chapter 2
Description on Study Area, Groundwater Sampling & Microbial Analysis

Chapter 3
Seasonal Variation of Microbial Quality

Chapter 4
Health Risk Assessment from Enteropathogens

Chapter 5
Association between Diarrhoea Occurrence and Groundwater Microbial Quality

Chapter 6
Summary of Research

Appendices

List of Figures

List of tables

CHAPTER 1

INTRODUCTION

1. 1 Background

1.1.1 Status of piped water supply services in Asian region

Many international organizations are separately or jointly putting effort to reduce water scarcity that the developing parts of the world are facing. Focusing on Asia and Pacific, Asian Development Bank (ADB) formulated and initiated 'Water for All' policy in 2001. Likewise United Nations (UN) initiated several Millennium Development Goals (MDGs) and one of them was to halve, by 2015, the proportion of the population without sustainable access to safe drinking water and basic sanitation (Goal 7 Target10 c). These efforts have resulted in 90% coverage of use of improved drinking water sources in South Asia by 2010 which was 18% increment in the level in 1990. However, the increments were basically made in other improved water sources rather than piped water. In South Asia, 65% of population use other improved sources than pipe source (25%) (UNICEF/WHO 2012). According to report by ADB, the number of people with a tap in the house (23%) lags significantly behind the overall MDG figures for improved water supply (91%) in South Asia (ADB/APWF 2013). Moreover, the data for piped water supply did not include duration of supply i.e. whether the supply is 24 hours a day and 7 days per week or intermittent supply (ADB/APWF 2013). According to a report on water in Asian cities published by ADB, the cities in which none of the population had 24 hours piped water availability were Dhaka, Karachi and Kathmandu whereas it was 1% and 60% in Delhi and Colombo respectively (Andrews & Yniguez 2004). These findings are clearly highlighting that in this region, water sources other than piped water constitute bigger proportion of household water.

1.1.2 Groundwater use in Asian region

Other improved sources consist of groundwater (tube well / protected bore well/ protected dug well), protected spring and rain water while unimproved sources include unprotected dug well, vendor's tanker water, unprotected spring water, bottled water, and surface water (WHO/UNICEF 2006). Among these water sources, groundwater is more accessible irrespective of season than springs, surface and rain water and is cheaper than bottled water and vendor's tanker water. Shallow groundwater wells can be installed close to where water supply is needed and individual can construct, operate and control their own supply in their own land. So, shallow groundwater is extensively used as small-scale use such as private self-supply at household level. Private self-supply is greatly practiced by urban dwellers as ‘coping-strategy’ against partially or highly inadequate municipal water supply (Foster et al. 2010). In Asian countries such as Bangladesh, China, India, Indonesia, Nepal, the Philippines, Thailand and Vietnam, groundwater served half of the potable water (Morris et al. 2003). It has been the major source for drinking and domestic activities mainly for the poorest urban households in many Asian countries including India and Bangladesh (IIED 2010). But, because groundwater is used as ‘private self-supply’ by households, it is often excluded from official statistics and is usually taken as granted by government officials and society.

1.1.3 Groundwater use in the Kathmandu Valley

The Kathmandu Valley being capital city is the most urbanized centre of the country. The city has seen extensive population growth which increased from 1.6 million in 2001 to 2.5 million by 2011 and the population growth rate of 5.2% was one the highest in South Asia (CBS 2012). Rapidly grown population has water demand of 320 million liters per day (MLD) but the water supplying agency could only provide 106 MLD and 76 MLD in wet and dry seasons respectively (KUKL 2010). In order to provide water to all connections despite of huge water deficit, the agency can supply water intermittently to the households. None of the municipal areas in the valley are receiving piped water 24 hours supply per day while most of them were receiving <4 – 7 hours per week (ADB 2010). Therefore, like in many Asian cities, alternative water sources constitute large proportion of domestic water use in the valley.

Among the alternative water sources in use in the valley, 52% of households use groundwater, 10% use stone spout, 1% use river, 27% use rainwater, 17% use bottled or jar, 8% use vendor’s tanker and 4% use other sources (ADB 2010). It is clear, from the above information, that groundwater is the most used alternative water sources in the valley among many others although the purposes of use could be different. A household survey revealed that 7.6% of the population used groundwater as a source of drinking water in the valley (CBS 2005). So, basically groundwater is being used for the purposes that need larger volume of water such as bathing, laundry, gardening etc.

According to Yoden (2012), the households which have private wells in their compounds rely on groundwater heavily. Although use of water sources such as river and rain are influenced by season, there is no big changes in the percentage of households that use groundwater in the wet season (48%) and in dry season (55%) (Yoden 2012). Therefore, in the Kathmandu Valley, groundwater is serving as easily accessible and inexpensive source of water at the household level.

In the Kathmandu Valley two types of groundwater wells are usually installed; dug and tube wells. Dug wells are hand excavated water wells that are usually cased with bricks or concrete rings masonry with a diameter of 1-2 m dug below the water table (Maharjan 2005). The dug wells may or may not be covered by lid and water is extracted by bucket or hand pump. Tube wells are wells made by drilling iron pipe into the ground and hand pump is attached to it to extract water.

1.1.4 Groundwater microbial pollution

Water pollution is a common problem in developing countries and contamination of groundwater is not an exception although it is usually perceived to be pristine and abundant. Groundwater has been widely reported to be contaminated with faecal indicator bacteria in many Asian countries such as in Bangladesh (Luby et al. 2007; Fergurson et al. 2011; Van Geen et al. 2011; Wu et al. 2011), Sri Lanka (Barthiban et al. 2012), Combodia (RDI 2008; Uy et al. 2010), and India (Krishnan et al. 2007). In Nepal, groundwater has been reported to be extensively contaminated with faecal indicator bacteria back in 1997 (Jha et al. 1997). Since then, numerous similar researches have been conducted till date and all have reported existence of severe faecal contamination in groundwater of the valley (Dongol et al. 2005; Maharjan 2005; Prasai et al. 2007; Warner et al. 2008; Pant 2011; Pujari et al. 2012). In almost all of these studies, minimum of 45% and maximum of 86% of the samples showed faecal contamination, given sample size greater than 50. Faecal contamination of water source indicates presence of human pathogens. Haramoto et al. (2011) identified human pathogens such as *Cryptosporidium* spp., *Giardia* spp., Human Adenovirus and Norovirus, (Haramoto et al. 2011). Likewise, Tanaka et al. (2012) identified multidrug resistant strains of opportunistic pathogen, Acinetobacter (Tanaka et al. 2012).

1.2 Statement of purpose

Microbial contamination of water can lead to transmission of water borne diseases. Once water gets contaminated with human or animal faecal material, consumption of such water sources leads to infection (Figure 1.1). Use of contaminated water for drinking, cooking, brushing teeth, washing etc. could all cause infection. Water borne diseases occur worldwide and are major causes of mortality and morbidity along with considerable social and economic impact (Pedley and Pond 2003). Water, sanitation and hygiene accounted for 4% of all deaths and 5.7% of total disease burden globally (Pruss et al. 2002) including diarrheal disease.

Diarrhea is the second leading cause of healthy time lost due to illness worldwide (Mathers et al. 2008). In Southeast Asia alone, diarrhea is responsible for 8.5% of all deaths and 38% of death in children of <5 yrs of age (WHO/UNICEF 2009). In Nepal, incidence of diarrhea among the children increased from 378 per 1,000 in 2007 to 598 per 1,000 in 2009 (MoHP, 2008/2009; MoHP, 2009/2010). Diarrhea covered 59% and 30% of total hospital cases of whole country and the Kathmandu Valley respectively and there is high possibility of an outbreak of diarrhea every year in the valley (MoHP 2009/10). Diarrheal diseases (Typhoid, Acute gastroenteritis, Ameboic dysentry, Bacillary dysentery and Cholera) constitute 69% of total water borne diseases in the valley (MoHP 2009/10). Diarrhoeal occurrence is more common when there is a shortage of clean water for drinking, cooking, cleaning and basic hygiene. In developing countries 94% of the diarrheal disease burden was associated with risk factor like unsafe drinking water and poor sanitation and hygiene (WHO/UNICEF 2006).

As detailed in the previous sections, groundwater of the valley has been severely contaminated with faecal indicator bacteria and human pathogens. Hence, microbial pollution of such widely used water source might have serious public health impact in the valley. However, studies on health impact from groundwater microbial quality as well as studies on examining different dynamics of microbial pollution such as spatial / temporal variations have been lacking. Therefore, exploring and analyzing several aspects of the microbial quality of the water source, in relation to public health effect and pollution control strategies could be helpful in curbing the threats to human health.

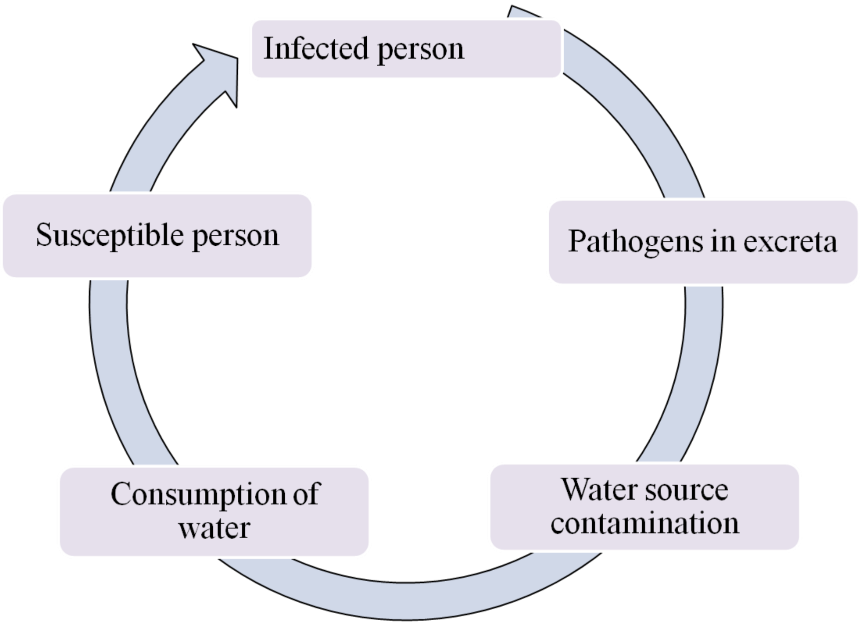


Figure 1.1 A pathway of water borne disease transmission

1.3 Objectives

The overall goal of this study is to propose measures for controlling and managing microbial pollution of groundwater from environmental perspective and for reducing associated health risks from household and social perspective in a developing country. In order to accomplish this goal, this study has taken the Kathmandu Valley as a case study with following specific objectives;

i.) To examine seasonal variation of microbial quality of groundwater and possible mechanisms. *(Chapter 3)*

ii.) To quantify risk of diarrhea from enteropathogens while using contaminated groundwater. *(Chapter 4)*

iii.) To verify the association between groundwater microbial quality and diarrheal occurrence at household level while controlling risk factors.*(Chapter 5)*

1.4 Dissertation outline

This dissertation consists of six chapters and this section gives brief description about individual chapters.

Chapter 1: Introduction

This chapter includes brief introduction about water supply status and importance of groundwater source in household water consumption in developing countries. It also highlights the gaps that current trend of studies have and the need of conducting research which help in understanding microbial pollution of groundwater and its effects on human health. On the basis of such needs this chapter introduces aim of this research. This chapter also includes brief dissertation outline and research frame work.

Chapter 2: Description on study area, groundwater sampling and microbial analysis

This chapter includes description of the study area and the methodology for conducting the targeted research. Each and every detail along with the references, wherever required, has been provided in this chapter in order to maintain integrity, transparency and reproducibility.

Chapter 3: Seasonal variation of microbial quality

This chapter examines seasonal variation of microbial pollution of shallow groundwater of the Kathmandu Valley as well as discusses two possible mechanisms for the variation.

Chapter 4: Health risk assessment from enteropathogens

Chapter 4 estimates the risk of diarrhoea from exposure to the current level of enteropathogens in shallow groundwater of the valley, either by drinking or by bathing pathway. It also discusses on the comparison of risk between well types and between enteropathogen types, on bathing as an important exposure pathway and on POU water treatment method as a methodological improvement of risk estimation especially for developing country setting. Finally, it estimates and discusses the relative impact of different parameters on risk from different enteropathogens.

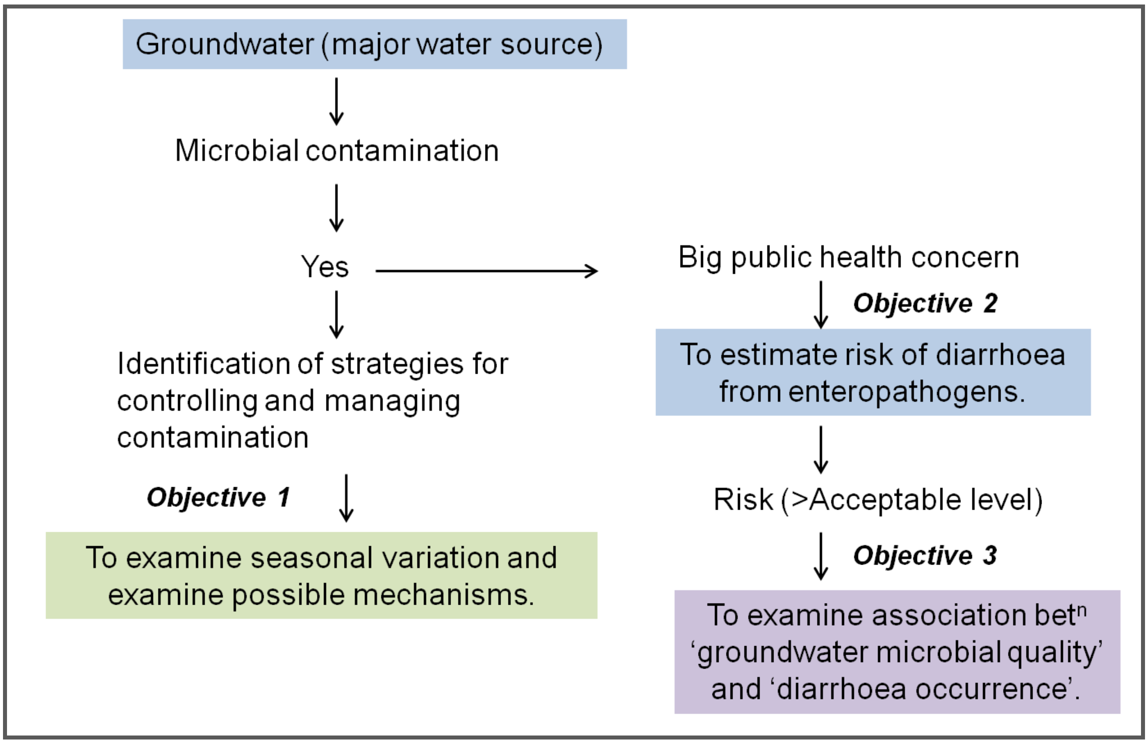
Chapter 5: Association between diarrhoea occurrence and groundwater microbial quality at household level

This chapter focuses on two major aspects. First is interpolation of groundwater microbial quality. This sub-chapter describes about kriging interpolation results and discusses on comparison between results obtained from different sample sizes. Second aspect describes the results of examining the association between diarrhoea occurrence and groundwater microbial quality using multivariable analysis. In this sub-chapter, association of other risk factors and diarrhoea occurrence have also been described and the possible explanations of the associations have been discussed.

Chapter 6: Summary of research

This chapter summarizes conclusions, generalization, contributions made through this study and further suggests the future research needs.

1.5 Research Framework



CHAPTER 2

DESCRIPTION ON STUDY AREA, GROUNDWATER SAMPLING & MICROBIAL ANALYSIS

2.1 Study area

2.1.1 Physiographic characteristics

Kathmandu Valley is situated in central hilly region of Nepal and consists of 85% of Kathmandu district, entire Bhaktapur district and 50% of Lalitpur (Patan) district. [A district is an administrative division consisting of cluster of 13 to 114 village development committees (VDCs). Each VDC has up to 9 wards and each ward is the smallest administrative unit of the country]. The valley has central flat part and altitude of 1300-1400 meters above sea level (masl). It is surrounded by mountains exceeding 2000 masl and is located between latitudes 27°32'13" and 27°49'10" north and longitudes 85°11'31" and 85°31'38" east (Pradhan et al. 2007).

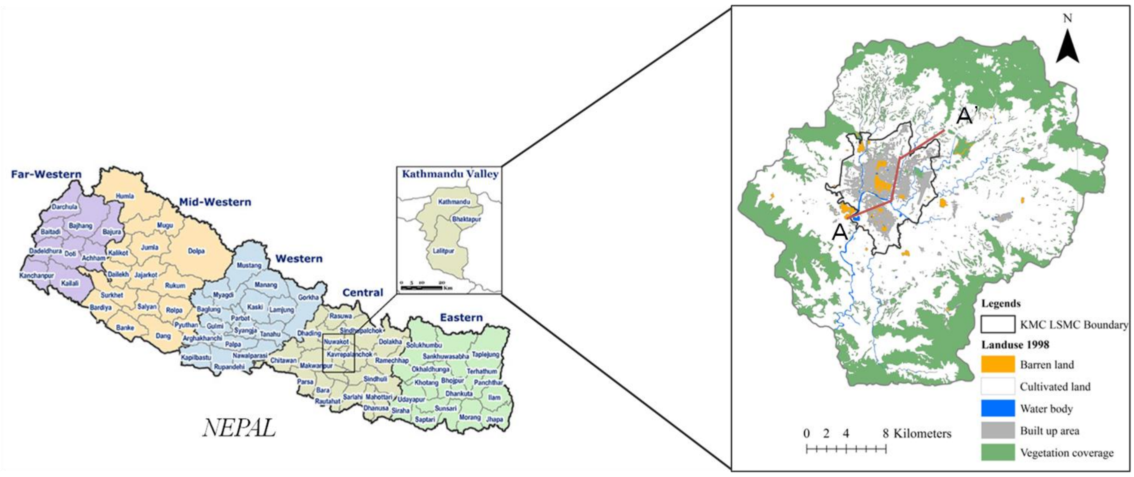


Figure 2.1 Map of the Kathmandu Valley

The valley is situated at the upper part of Bagmati River Basin (BRB). It is drained by the Bagmati River and its tributaries such as Balkhu, Bishnumati, Dhobi Khola, Manohara, and Nakhu. The Bagmati River leaves the valley at Chobhar, the south of the watershed. The watershed boundary covers approximately 664 km^2 areas (Acres International 2004). The groundwater basin within the watershed covers around half (i.e. 327 km^2) of the area.

By tradition, the aquifer of the valley basin has been divided into shallow and deep aquifer system (Figure 2.2). The shallow aquifer is composed of up to 50m of Quaternary arkosic sand, with some discontinuous interbedded silt and clay of the Patan and Thimi Formations (Yoshida and Igarashi 1984). This sediment is underlain by a clay aquitard that is up to 200 m thick in the western valley. The deep aquifer that lies beneath clay aquitard consisted of the Pliocene sand-and-gravel, with interbedded lignite, peat, and clay (Jha et al. 1997). Groundwater from shallow aquifer is drawn from dug wells and tube wells (Gurung et al. 2007). Recharge of the shallow aquifers occurs mostly along basin margin directly from precipitation and from small rivers (Gurung et al. 2007). Deep aquifer is tapped by deep tube wells and is recharged in the northeast part of the valley where thick clay layers are not present (Warner et al. 2008).

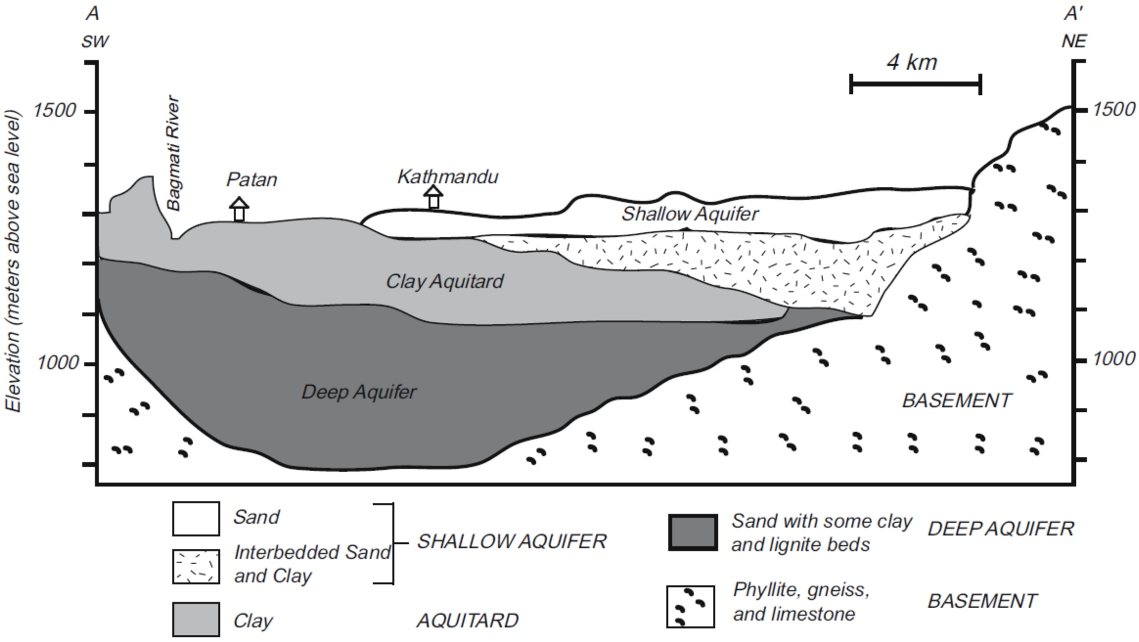


Figure 2.2 Cross section through the Kathmandu Valley *(Source: Warner et al. 2008)*

2.1.2 Climate

The valley lies in temperate climate zone with average temperature of 18^{o}C, the mean minimum temperature in the coldest month (January) is 1 to 2^{o}C and freezing are rare (Sakai 2001). The Kathmandu Valley experiences four distinct seasons during a year; pre-monsoon (March – May), monsoon (June – September), post monsoon (October – November) and winter (December – February) (Aryal et al. 2008). Average annual rainfall is 1755 mm (Acres International 2004) with altitudinal variation from about 1300 mm in the valley floor to about 3000 mm in the mountains surrounding the valley (HMG/N 2005). Eighty percentage of the total rainfall occurs in monsoon season (JICA 1990). Winter months remain mostly dry with occasional precipitation in the form of winter rains caused by westerly cyclones.

2.1.3 Population

By 2011, national population reached to 26.6 million with annual growth rate of 1.40 percent. As per the census results, out of total population 17% (4.52 million) reside in urban area. The Kathmandu Valley is the largest urban centre of the country and has been center of administration, economy, education and politics. Kathmandu district has the highest population density (4408 per sq. km) and is around 30 times higher than national population density (181 per sq. km). The district has recorded highest decadal population growth (60.93%) compared to that of all Nepal (14.99%) (CBS 2011). The population of the Kathmandu Valley rose to 2.51 million by 2011 with an annual growth rate of 3.65% which was 1.6 million in 2001 (CBS 2011).

2.1.4 Water demand and sources in use

Rapidly grown population has water demand of 320 million liters per day (MLD) but the water supplying agency could provide 106 MLD and 76 MLD in wet and dry seasons respectively (KUKL 2010). In order to provide water to all connections despite of huge water deficit, the water supplying agency provides intermittent water to the households. None of the municipal areas in the valley are receiving piped water 24 hours per day while most of them were receiving <4–7 hours per week (ADB 2010). Therefore, alternative water sources constitute large proportion of domestic water use in the valley. Among the alternative water sources in use in the valley 52% of households use groundwater, 10% use stone spout, 1% use river, 27% use rainwater, 17% use bottled or jar, 8% use vendor’s tanker and 4% use other sources (ADB 2010). It is clear from the above information that groundwater is the most used alternative water sources in the valley among many others although the purpose of use could be different.

2.2 Groundwater sampling

Groundwater samples were collected from dug wells and tube wells in January 2009 and 2012, August 2009 and 2010, May 2011 and September 2012. According to the seasons in which these sampling months fall, we denoted January as the dry season, May as the pre-monsoon season and August and September as the wet season. Hence, the sampling periods were denoted by dry or wet season followed by the year in the subsequent sections; ‘Dry-2009’ for January 2009, ‘Wet-2009’ for August 2009, ‘Wet-2010’ for August 2010, ‘Pre-monsoon-2011’ for ‘May 2011’, ‘Dry-2012’ for January 2012 and ‘Wet-2012’ for September 2012. The number of samples collected in each season is given in Table 2.1. Additionally, three wells among them were monitored monthly for nearly a year from October 2011 to September 2012. Figure 2.3 showed geographical locations of all the sampled wells.

From each well, water was purged for 3-5 minutes until hydrogen ion concentration (pH), temperature and electrical conductivity (EC) became stable. Then sample was taken in polythene bottles for microbial analysis. Water is directly collected with clean buckets from the dug wells that were without hand pumps. Before collecting water sample, the clean bottles were rinsed properly. After collecting sample the sample bottles were then immediately stored in ice box. For the dug wells, the depth of water table below ground was also measured.

Table 2.1 Sample sizes by season, year and well types

Season	**Year**	**Dug well (n)**	**Tube well (n)**
Dry	2009	15	24
Dry	2012	18	23
Wet	2009	16	20
Wet	2010	16	20
Wet	2012	15	15
Pre-monsoon	2011	19	25

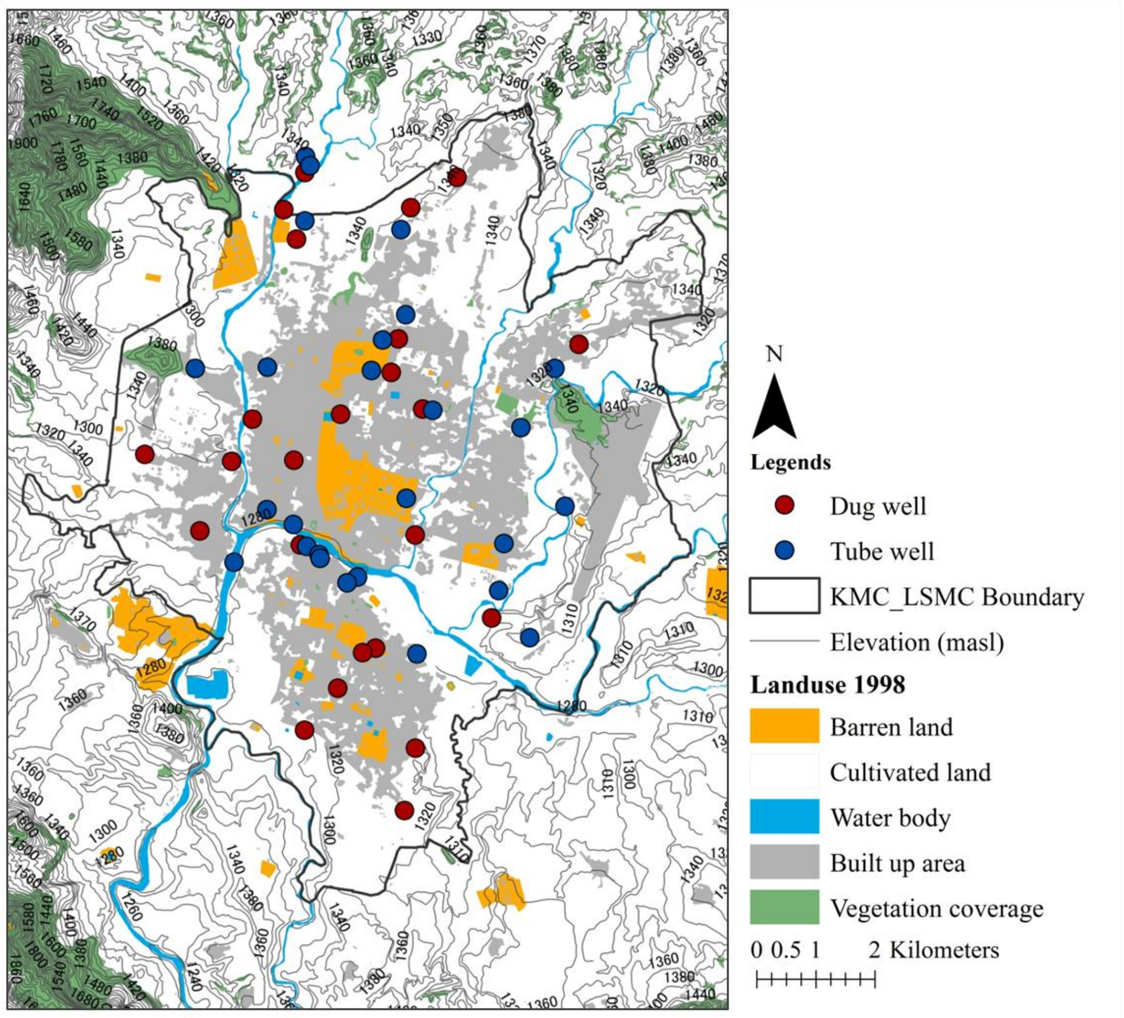


Figure 2.3 Distribution of dug and tube wells accessed for groundwater sampling

2.3 Microbial analysis

2.3.1 Escherichia coli *(E. coli)* and Total coliform detection:

The microbial pollution in the groundwater of the valley was measured using coliform; *E. coli* and total coliform. *E. coli*, commensal of numerous warm blooded animal digestive tract, is the most appropriate coliform to indicate fecal contamination of warm blooded animal (Payment et al. 2003). *E. coli* and total coliforms were measured in Dry-2009 by Environmental Protection Agency (EPA)-approved membrane filtration method using Hach m-ColiBlue24 Broth. The groundwater samples were filtered through 0.45µm pore sized filter and organisms were grown in Hach's m-ColiBlue24 Broth after incubating for 24 hours at 35±0.5^{0}C. The red and blue colonies indicating total coliform and blue colonies only indicating *E. coli* were counted and results were presented as colony forming unit (CFU) per 100 ml.

From Wet-2009 onwards, EPA-approved IDEXX Quanti-Tray method (USA) using Colilert reagent, was used. In this method, Colilert reagent was used for the simultaneous detection and confirmation of total coliforms and *E. coli* in water. After mixing the reagent, the solution was poured into the tray and the tray was sealed and incubated for 24 hours at 35 ± 0.5^0C. After incubation, wells in the tray producing yellow color (total coliform) under sun light and blue color (*E. coli*) under UV light were counted. We referred to the maximum probable number (MPN) table to determine MPN of total coliform and *E. coli* in the 100 ml of the sample. There was a good correlation between membrane filtration and Quanti-Tray methods for the detection of *E. coli* and total coliform concentration.

The microbial analysis was carried out as soon as the field sampling was over. It was done in laboratory of Center of Research on Energy Environment and Water (CREEW) in the Kathmandu Valley, Nepal. According to the protocol of the above mentioned procedures, the lower detection limit is 1 MPN/100 ml and the value for not detected (ND) samples is < 1 MPN/100 ml.

2.3.2 *Cryptosporidium* oocyst and *Giardia* cyst detection:

Analysis of *Cryptosporidium* oocyst and *Giardia* cyst was carried out for 22 dug wells and 15 tube wells samples during the wet seasons (Wet-2009 & Wet-2010) only. *Cryptosporidium* oocysts and *Giardia* cysts were detected by immunomagnetic separation using Dynabeads GC combo (Invitrogen, Carlsbad, CA, USA) and an immunofluoresence assay using Easy Stain (BTF, North Ryde, Australia).

There are three sequential steps in protozoa detection; concentration, immunomagnetic separation and immunofluoresence assay.

Concentration:

In this step protozoa (oo)cysts present in 1 L of groundwater sample were separated from the water sample. In 1 L of sample 2.5 mol/l $MgCl_2$ was added and the $MgCl_2$-supplemented water was then filtered through a mixed cellulose ester membrane (pore size 0.45μm, diameter 47 mm; Millipore, Billerica, MA, USA) using a vacuum pump system. Then the filter membrane was kept inside 50 ml plastic tube and 12 ml of elution buffer was added. It was followed by vigorous vortexing of the membrane. Except 1 ml supernatant, remaining 11 ml was used for protozoa detection.

Immunomagnetic separation:

Ten milliliter of sample, 1 ml of 10xSL^{TM} – Buffer A and 1 ml of 10xSL^{TM}-Buffer B were added in a flat sided L-10 tube. Then, 100 µml of Dynabeads® anti-*Cryptosporidium* and 100µml of Dynabeads® anti-*Giardia* were also added to the tube. The beads were thoroughly re-suspended by inverting the tube. Then the tube was affixed in a rotating mixer and rotated at 15-20 rpm for 1 hr at room temperature. After that the tube was removed and placed in a magnetic separator (MPC^{TM} – S) with the flat side of the tube facing towards magnet and then gently swung in 90 degree angle for 2 min. Then with the magnet in upright position cap was removed and solution was poured immediately. Again 10 ml of Phosphate buffer solution (PBS) was added and repeated the previous step two times. L-10 tube was removed from the magnet and 0.5 ml of SL-Buffer A was added into the tube and mixed gently then liquid was transferred into 1.5 ml centrifuge tube. For dissociating Dynabeads® - Cysts/Oocysts complex 50 µL of 0.1N HCl was added to the tube and applied vortex for 10 sec. The tube was then placed in MPC^{TM}-S with magnetic strip and waited for a minute. And pour solution in another micro-centrifuge tube with 10 µL of 1N NaOH. In previous centrifuge tube 50µL of 0.1N HCl was again added and vortexing was done. The tube was then again placed in MPC^{TM}-S with magnetic strip and waited for a minute. Then the solution was poured in the previous micro-centrifuge tube. Now the micro-centrifuge tube has 110 µL of the solution.

Immunofluorescence assay:

The 110 µL purified sample was passed through a hydrophilic polytetrafluoroethylene (PTFE) membrane (pore size 1.0 µm, diameter 25 mm; Advantec), followed by fluorescent staining of protozoa on a membrane using EasyStain (BTF, North Ryde, Australia) according to the manufacturer’s protocol. A FluoView FV1000 laser scanning confocal microscope (Olympus, Tokyo, Japan) was used to count the number of *Cryptosporidium* oocysts (round-shaped, 4–6 µm diameter) and *Giardia* cysts (oval-shaped, 5–8 µm diameter and 8–12 µm width).

CHAPTER 3

SEASONAL VARIATION OF MICROBIAL QUALITY

Seasonality is one of the many factors affecting groundwater microbial quality and it should be understood beforehand in order to develop groundwater pollution control and management strategies and then improve the quality to maintain public health safety. This chapter describes the necessities of seasonal variation in microbial quality of shallow groundwater, describes the results and discusses the possible mechanism of such variation.

3.1 Introduction

Seasonality is one of the many factors affecting groundwater quality and it should be understood beforehand in order to maintain public health safety. Especially in resource poor settings, the season should be identified to execute efficient pollution management strategy with limited financial and human resources.

The Kathmandu Valley has distinct dry and wet seasons. The dry season begins in October and ends in May; the wet season begins in June and ends in September. Average annual precipitation is 1400 mm, 80% of which occurs during the wet season (Acres International 2004). This intense wet season influences the chemical parameters of the valley's groundwater, which has a significantly higher biochemical oxygen demand (BOD) and chemical oxygen demand (COD) during the wet season than the dry season (Kannel et al. 2008).

Seasonal influences on the microbial contamination of groundwater can be site-specific. In Northern Mozambique, microbiological contamination of shallow groundwater increased with the onset of rainfall (Godfrey et al. 2005). Jha et al. (1997) and Dongol et al. (2005) detected higher microbial concentrations in the groundwater during the wet season than the dry season in Nepal. Whereas in Sierra Leone, low rainfall and diminishing water recharge during the dry season caused a lower dilution level and produced higher concentrations of faecal indicator bacteria (Wright 1986). However, in case of the valley the seasonal variation in the microbial quality was still unclear and the possible mechanisms underlying those variations were yet unexplored. Hence, it was very essential to evaluate the urgency of implementing countermeasures against microbial pollution of shallow groundwater and identifying the season to be targeted.

Hence our objective was to examine seasonal variation in microbial quality of shallow groundwater and to examine possible mechanisms of such variation.

3.2 Methodology

3.2.1 Groundwater samples and microbial analysis

Groundwater sample collection and microbial analysis were described in *section 2.2*. The microbial parameters considered were *E. coli* and total coliform. Additionally, two wells (DSG18b and DSG37) with very large fluctuations in *E. coli* concentration and one well (DSG24) with small fluctuations were monitored monthly for nearly a year from October 2011 to September 2012. For these monitoring wells, microbial quality as well as water level below the ground surface was measured.

3.2.2 Wastewater loading

Wastewater loading was calculated in order to examine its influence on shallow groundwater microbial pollution. For civic administration, KMC and LSMC are further divided into many administrative wards. For this study, cumulative figure of wastewater loading (L) from two types of population (resident population and floating population) was considered. Per day wastewater loading was calculated for each administrative ward. Floating population for each administrative ward was calculated from total number of different types of institutions (office, hospital, hotel, school etc.) and probable number of people linked to each institution (KMC, 2004). Domestic wastewater production was calculated as 85% of water uses (75 liters per capita per day) (Shukla et al., 2011). According to EPA Design Manual "Onsite Wastewater Treatment and Disposal Systems", wastewater generated from office and hospital was 10.6 gallon per day per person (gpd), from hotel was 50 gpd, from school was 15.9 gpd (USEPA, 1980). For LSMC, wastewater loading was calculated based on resident population only because data on number of institutions was unavailable.

In this study, ward wise comparison of wastewater loading and microbial quality of groundwater was compared in order to obtain essence of relationship between possible source and contamination.

3.2.3 Rainfall data

Rainfall data was obtained from Department of Hydrology and Meteorology (DHM), Government of Nepal. It comprised of daily rainfall record from year 2000 until 2009 from 9 rain gauge stations in the Kathmandu Valley. For the purpose of our analysis, we first calculated daily average of nine rain gauge stations for each year. So we had average daily rainfall for each year. Then, we calculated cumulative monthly rainfall for each year and then finally we calculated average monthly rainfall from 10 years data. To analyse the relationship between rainfall and the microbial concentration in groundwater, the average rainfall in December and January was considered to be the dry season's monthly average; that in April and May as the pre-monsoon season's monthly average; that in July and August as the wet season's monthly average.

3.2.4 Statistical analysis

In order to examine seasonal variation within well types, yearly comparison between two seasons were assessed. Mann–Whitney U test was used to compare Dry-2009 with Wet-2009 and Dry-2012 with Wet-2012. Further, we combined two dry season data (Dry-2009 & Dry-2012) as one and two wet season data (Wet-2009 & Wet-2010) as one because of the observed stability of microbial parameters over different years and then the combined data were compared for existence of seasonal variation. For this, we used independent samples t-test. Pearson's correlation coefficient was used to identify the associations between wastewater loadings and *E. coli* concentrations as well as between groundwater levels and *E. coli* concentrations in the three monitoring wells. These analyses were performed using the Statistical Package for Social Studies version 21 (SPSS Inc., USA). In statistical analysis, the values (< 1 MPN/100ml) for ND samples are replaced with lower detection limit (1 MPN/100ml).

3.3 Results

3.3.1 Descriptive statistics of microbial concentration of groundwater in dry and wet seasons

The minimum, maximum and mean concentration and detection rate for *E. coli* and total coliforms are shown in Table 3.1. The detection rate and concentration of total coliform were higher than that of *E. coli* both in dry and wet seasons and in both types of wells. According to WHO guideline for drinking water, water from overall 90% of the wells were not fit for using for drinking purpose. The mean *E. coli* and total colifrom concentration for the combined wet season was higher than that for the combined dry season for both types of wells. In addition, the recorded maximum *E. coli* concentration was higher in the wet season than in the dry season in both types of wells. Similar results were obtained for total coliforms. In the wet season, the maximum total coliforms concentration reached to the order of 10^5.

Table 3.1 Descriptive statistics of the microbial parameters

				E.coli conc. (MPN/100 ml)				Total coliform conc. (MPN/100 ml)			
Well type	Season	Year	N	Mean	Min	Max	Detection rate (%)	Mean	Min	Max	Detection rate (%)
Dug	Dry	Combined	33	1.7×10^2	<1	2.3×10^3	67	2.7×10^3	2.9×10^1	1.9×10^4	100
		2009	15	3.6×10^2	<1	2.3×10^3	80	2.9×10^3	2.9×10^1	1.9×10^4	100
		2012	18	2.4×10^1	<1	2.2×10^2	63	2.8×10^3	6.3×10^1	1.2×10^4	100
	Wet	Combined	32	2.9×10^2	<1	6.9×10^3	94	2.5×10^4	4.4×10^1	2.9×10^5	100
		2009	16	1.2×10^3	<1	6.9×10^3	94	2.4×10^4	4.4×10^1	2.9×10^5	100
		2010	16	7.7×10^2	<1	3.4×10^3	93	2.5×10^4	8.7×10^2	2.6×10^5	100
		2012	15	2.6×10^3	<1	2.0×10^4	93	2.3×10^4	2.4×10^2	2.4×10^5	100
Tube	Dry	Combined	47	1.7×10^1	<1	5.7×10^2	57	3.8×10^2	2.0×10^1	4.0×10^3	100
		2009	24	3.2×10^1	<1	5.7×10^2	57	4.9×10^2	4.0×10^1	4.0×10^3	100
		2012	23	1.2×10^1	<1	1.1×10^1	26	2.7×10^2	2.0×10^1	1.9×10^3	100
	Wet	Combined	40	2.0×10^1	<1	6.9×10^2	50	7.1×10^2	<1	9.2×10^3	90
		2009	20	3.8×10^1	<1	6.9×10^2	40	4.4×10^2	<1	2.4×10^3	90
		2010	20	2.0×10^1	<1	1.2×10^1	58	9.8×10^2	<1	9.2×10^3	90
		2012	15	1.7×10^2	<1	1.1×10^3	60	1.6×10^3	<1	1.0×10^4	93

Max: Maximum; Min: Minimum; Combined: seasonwise data combination; For Dry-2009 unit was CFU/100 ml

3.3.2 Spatial distribution of *E. coli* concentrations

To understand the mechanism of microbial pollution of groundwater simple analysis on its spatial distribution was conducted during the dry and wet seasons and its relationship with wastewater loading was examined. While the distribution patterns of the two seasons were different, microbial concentrations in neighbouring wells were heterogeneous, showing no particular groupings (Figure 3.1 & 3.2). Wastewater loading was estimated for each administrative ward in KMC and LSMC, and examined its correlation with groundwater microbial concentrations. The Pearson's coefficient for correlation of wastewater loading with *E. coli* concentration in dug wells only, tube wells only and both wells together were -0.17, 0.21 and -0.10 respectively. These results showed no particular relationships between wastewater loadings and microbial quality of groundwater.

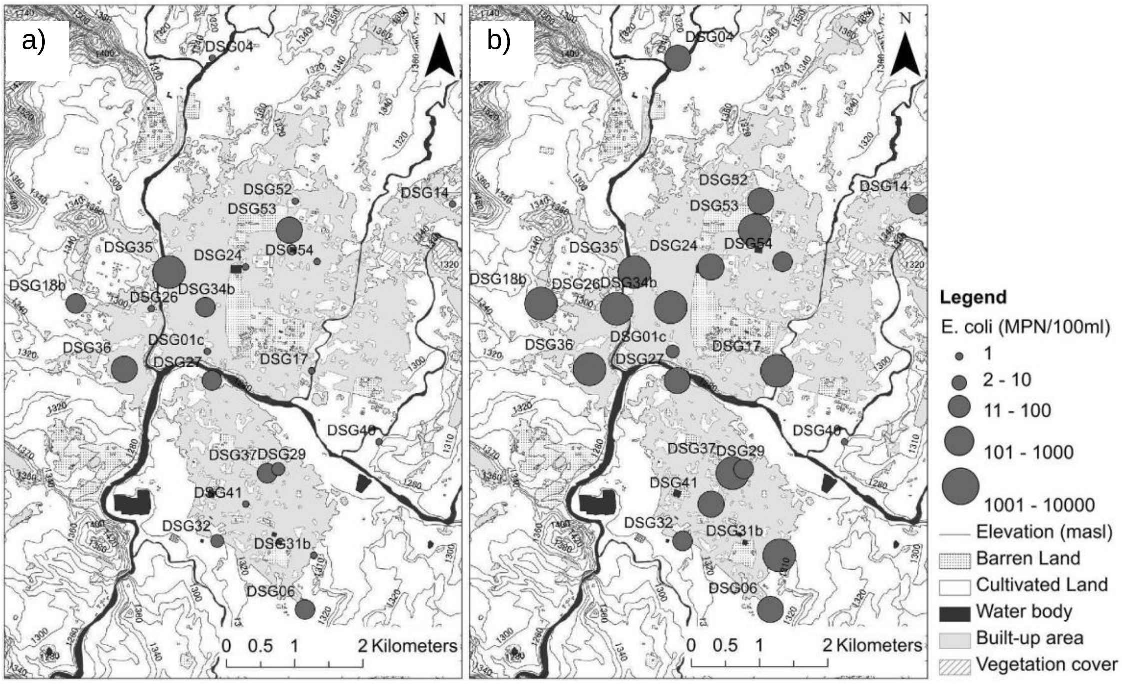


Figure 3.1 Spatial distribution of *E. coli* concentrations in dug wells a) dry season and b) wet season

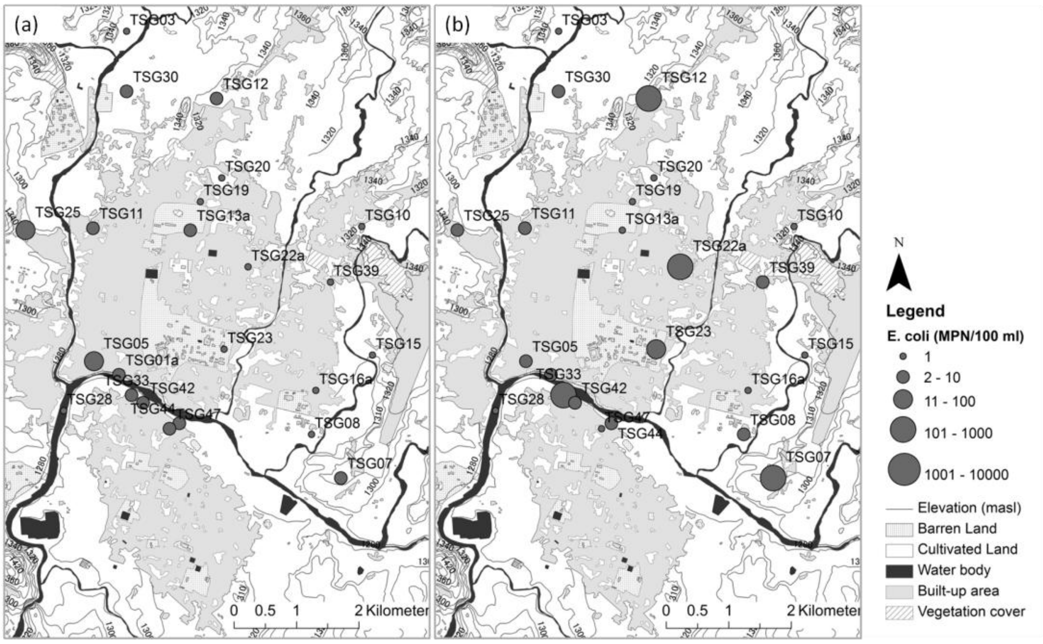


Figure 3.2 Spatial distribution of *E. coli* concentrations in tube wells a) dry season and b) wet season

3.3.3 Seasonal variations in microbial concentrations in dug well

The *E. coli* concentrations in Wet-2009 and Wet-2012 were significantly higher than those in Dry-2009 ($p < 0.05$) and in Dry-2012 ($p < 0.01$) respectively (Figure 3.3a). The mean *E. coli* concentration in the combined wet season was significantly higher than that in the combined dry season ($p < 0.01$). Similarly, the concentration of total coliforms in Dry-2009 and Dry-2012 were significantly lower than that in Wet-2009 ($p < 0.05$) and in Wet-2012 ($p < 0.01$) (Figure 3.3b). The mean concentration of total coliforms in the combined wet season was significantly higher than that in the combined dry season ($p < 0.05$).

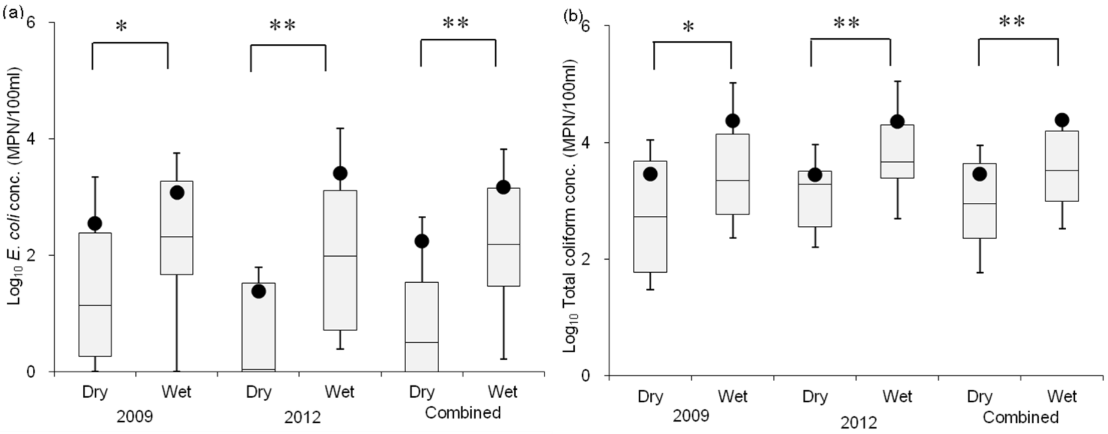


Figure 3.3 Concentrations of a) *E. coli* and b) Total coliform in dug wells. Box: inter-quartile range; dot: mean; high & low lines: 90^{th} & 10^{th} percentiles; *: p-value<0.05; **: p-value<0.01.

3.3.4 Seasonal variations in microbial concentrations in tube well

Different from dug wells, *E. coli* concentrations in tube wells as well were significantly higher in Wet-2012 than in Dry-2012 ($p < 0.05$) only (Figure 3.4a). However, data of year 2009 and the combined data did not show any significant differences. In case of total coliform, there were not any significant differences between concentrations in dry and wet seasons (Figure 3.4b). For both *E. coli* and total coliform concentration, most of the comparison types did not show significantly higher values in wet season compared in dry.

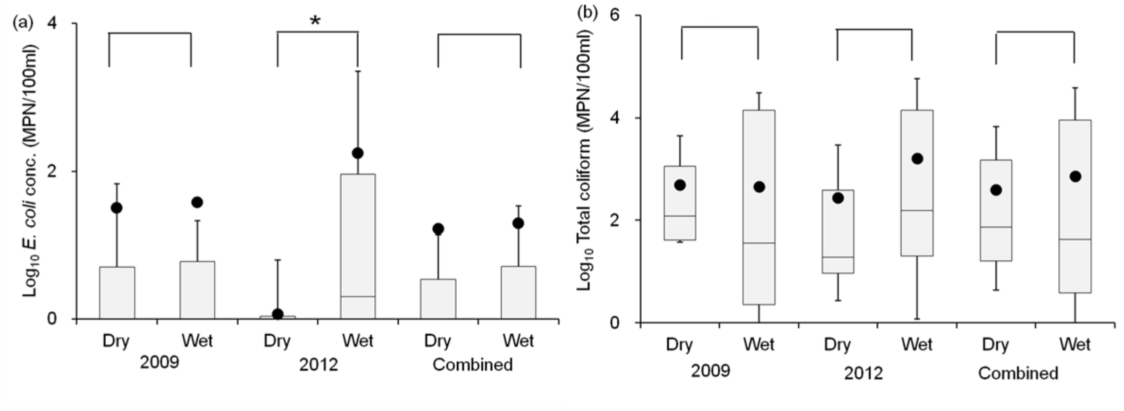


Figure 3.4 Concentrations of a) *E. coli* and b) Total coliform in tube wells. Box: inter-quartile range; dot: mean; high & low lines: 90th & 10th percentiles; *: p-value<0.05; **: p-value<0.01.

3.3.5 Relationship between rainfall and microbial concentration in groundwater

It is a natural phenomena that the average rainfall amounts, obtained from 10 years data, were lowest in dry (10 mm), increased in pre-monsoon (112 mm) and highest in wet season (422 mm) respectively (Figure 3.5). Similar to the trend of seasonal variation in rainfall, the average *E. coli* concentration (MPN/ 100 ml) in shallow groundwater were lowest in dry (dug well: 175; tube well: 17), increased in pre-monsoon (dug well: 811; tube well: 9) and became highest in wet season (dug well: 1490; tube well: 20).

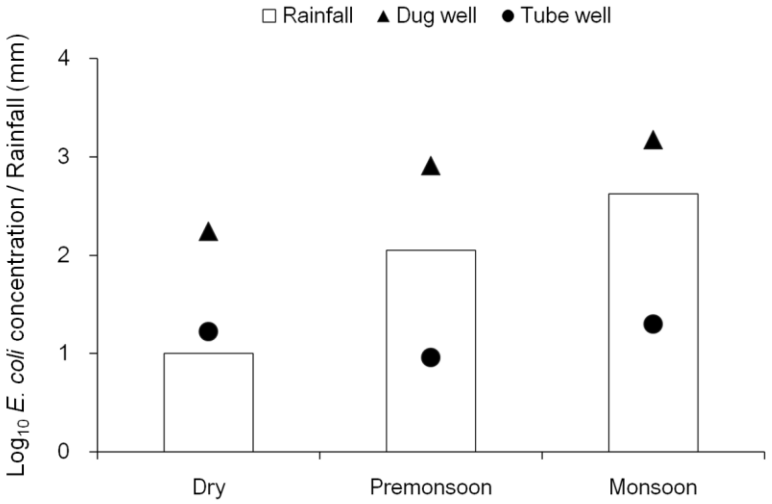


Figure 3.5 Variations in monthly rainfall and *E. coli* concentrations in the dry, pre-monsoon and wet seasons.

3.3.6 Results from monitoring wells for water level and microbial concentration

Three monitoring dug wells, DSG18b, DSG24 and DSG37, were examined for about a year for parameters such as water level below water surface and *E. coli* concentration. *E. coli* concentrations and groundwater levels simultaneously varied in all the three wells except during February and September in DSG18b and August and September in DSG24 (Figure 3.6).

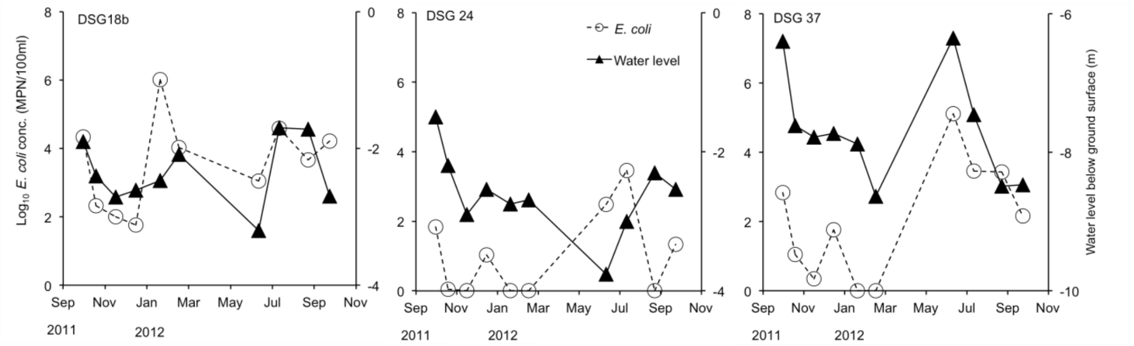


Figure 3.6 Monthly variations in *E. coli* concentrations and water levels below the ground surface. Circles: *E. coli* concentration; triangles: water levels.

We also examined the correlation between water levels and *E. coli* concentration on seasonal basis (Dry: October to May; Wet: June to September). Except for DSG24 in the wet season, all the three wells showed positive and moderate correlation (correlation coefficient, r = 0.48–0.88) (Table 3.2). DSG18b showed least correlations between these parameters whereas DSG24 showed moderate to strong and DSG37 showed strongest correlations. However, the significance level was achieved only for the correlation in DSG37 for dry season.

Table 3.2 Correlation between *E. coli* concentration and water level below ground surface

Seasons	Monitoring Wells	N	Correlation coefficient (r)	p-value
Dry	DSG18b	6	0.48	0.33
	DSG24	6	0.80	0.06
	DSG37	6	0.87	0.03
Wet	DSG18b	4	0.61	0.39
	DSG24	4	-0.69	0.20
	DSG37	4	0.88	0.12

3.4 Discussion

3.4.1 Spatial distribution of microbial quality of groundwater

The spatial distributions of microbial concentrations in groundwater of the two seasons were different. But in both seasons microbial concentrations in neighbouring wells were dissimilar and apparently there were no particular clustering in whole study area. In urban area, wastewater is highly possible for groundwater faecal contamination but in our case there were no specific correlations between wastewater loadings and microbial concentrations in groundwater. It might be because, each ward had only one or two sampling stations and those samples might not be representative of the ward. Wastewater production values that we referenced from USEPA could be larger than that in Nepalese context and estimated wastewater loading might be very different from real quantity. In addition, wastewater loading could be poor proxy of mechanism of pollution. Our results could not explain the mechanism of pollution and of the observed spatial distribution. In future, spatial variation should be examined using techniques such as geographic information system (GIS).

3.4.2 Seasonal variation of microbial concentration groundwater

Seasonal variations of *E. coli* and total coliform concentrations were significantly established for dug wells in all the comparison types in this study. For tube well the variations were established for *E. coli* concentration in year 2012 only. This study, hence, confirmed that microbial concentration increases during wet season especially in tube well which indicated that there could be more health risk involved while using those sources for drinking and other household purposes particularly during wet season. In addition, these results also suggested prioritizing wet season, in resource poor settings, for forming and implementing pollution control strategies or simply raising awareness about groundwater pollution.

Similar increasing trends in groundwater quality parameters in wet seasons have been identified in Nepal (coliforms, BOD and COD) and in abroad (coliforms) in shallow wells (Table 3.3). Jha et al. (1997) reported increased faecal coliform concentrations in the shallow groundwater during the wet season in the Kathmandu Valley. Similarly, Dongol et al. (2005) detected the lowest microbial concentration during the dry season (December–February), an increased concentration in the pre-monsoon season (March–May) and the highest concentration in the wet season

(June–September). Microbial concentrations in shallow groundwater respond to rainfall (Godfrey et al. 2005). However, Jha et al. (1997) and Dongol et al. (2005) neither examined this relationship nor analysed seasonal variations statistically. Therefore to identify such possible mechanisms we did further analyses.

Table 3.3 Researches comparing groundwater quality parameters in dry and wet seasons

Author	Site	Type of well	Parameter	Dry season (mean/median)	Wet season (mean/median)	p - value*
Jha et al., 1997	Kathmandu, Nepal	Dug	Fecal coliforms (col/100ml)	1.3×10^2	4.4×10^3	NA**
Jha et al., 1997	Kathmandu, Nepal	Tube		1.4×10^2	8.8×10^1	NA**
Dongol et al., 2005	Kavre, Nepal	Dug	Total coliforms (MPN/100ml)	1.7×10^2	$> 1.8 \times 10^2$	NA**
Dongol et al., 2005	Kavre, Nepal	Dug	*E. coli* (CFU/100ml)	6.0×10^0	2.9×10^2	NA**
Kannel et al., 2008	Kathmandu, Nepal	Dug	BOD (mg/L)	1.0×10^1	1.9×10^1	< 0.01
Kannel et al., 2008	Kathmandu, Nepal	Dug	COD (mg/L)	1.7×10^1	3.3×10^1	< 0.01
Valenzula et al., 2009	ESJ Watershed. Chile	Dug	Fecal coliforms (CFU/100ml)	1.0×10^1	1.9×10^2	< 0.01
Present study	Kathmandu, Nepal	Dug	*E. coli* (MPN/100ml)	1.7×10^2	9.9×10^2	< 0.01
		Tube		1.2×10^1	1.7×10^2	< 0.01
Present study	Kathmandu, Nepal	Dug	Total coliforms (MPN/100ml)	2.7×10^3	2.5×10^4	< 0.01
		Tube		3.8×10^2	7.1×10^2	< 0.01

** Significance level for comparison between dry and wet seasons ** Not available*

3.4.3 Mechanisms of seasonal variations

a. Surface runoff

Valenzuela et al. (2009) assumed that the faecal microbes in shallow groundwater are transported by runoff during the wet season. But no direct intrusion of surface runoff in the wells was observed in our study. Most of the dug wells had concrete walls (> 0.3 m height) and tops sealed with concrete slabs. Therefore, we assumed two hydrological processes responsible for the seasonal variation: i) infiltration of contaminants and ii) change in the water level below the ground surface.

b. Infiltration of contaminants

In our study, both mean *E. coli* concentrations in the wells and mean amount of rainfall in the valley were the lowest during the dry season, increased during the pre-monsoon season and were the highest during the wet season (Figure 3.5). These results suggest that the microbial transport into the groundwater increases during high rainfall. Kannel et al. (2008) suggested that higher rainfall and river flow during the wet season may cause higher infiltration of contaminants. Their study analysed the influence of river water on groundwater pollution and located all sampling stations on the ‘river corridor’. But as the surface water levels in the rivers were lower than the water levels in the wells near the rivers, we expected to have little or no river water influence on groundwater quality. Therefore, between the two possible explanations for the infiltration of contaminants, high rainfall was likely responsible in our case. Groundwater table for our samples varied from 1 to 13 m below ground surface. Pandey and Kazama (2011) estimated that the hydraulic conductivity of the valley’s shallow aquifer ranges from 12.5 to 44.9 m/day. Considering the shallow aquifer’s hydraulic conductivity, the soil properties described by Kannel et al. (2008) and the water table of our wells, rainwater could reach the groundwater table within the same season.

It is true that most of the households have sewer connection and large part of waste water is flown out via sewer system. However, Shrestha (2011) reported that in urban area 21% of households use septic tank and 4% use pit latrine and in peri-urban areas more than 50% of households use onsite sanitation system. In the Kathmandu Valley, sewers are leaky because of improper construction (Nyachhon 2006). Moreover, septic tanks are improperly constructed and in older areas, pit latrines are still in use (Shrestha 2011). Therefore, it is likely that huge amount of wastewater is being leaked into the valley’s sub-surface. In the valley, storm drains and sewer lines carry mixtures of sewage and storm water and the drains and manholes frequently overflow that pollutes the land surface (Pradhan et al. 2007). In addition, poor solid waste management (Pradhan et al. 2007) results in the contamination of the land surface. Unlike the dilution mechanism described by Wright (1986), even during the wet season, these highly contaminated surfaces and sub-surfaces could undoubtedly increase the pollutant load.

This study could not examine the influence of river on groundwater quality. Therefore we would like to recommend for detailed study on groundwater flow and river water influence using groundwater modelling.

c. Change in the water level below ground surface

The examination of *E. coli* concentration variation along with that in water below ground surface was conducted with the help of dug wells (DSG18b, DSG24 and DSG37) only which were monitored for the parameters for about an year. However, since this examination was based on underground phenomena, we assumed that the mechanism could be similar to and results could be interpreted for tube wells as well.

E. coli concentrations and groundwater levels simultaneously varied in all the three wells except during February and September in DSG18b and August and September in DSG24 (Figure 3.6). In all the three wells, the correlation between water level and *E. coli* was moderate to strong (correlation coefficient, r = 0.48–0.88), except for DSG24 in the wet season. Dilution process could explain the result of DSG24 in wet season but could not explain all temporal variations for the three wells. This result indicates that all three wells may have undergone the same contamination process i.e. change in the water level below ground surface. As the water level rose, the groundwater may have reached the point of pollution in the sub-surface, and filtration of micro-organisms by soil layers may have been reduced.

Compared to DSG37, DSG18b and DSG24 were in less densely populated areas, and DSG18b was newly constructed. As water level and *E. coli* concentration significantly correlated only in DSG37, pollution sources in less populated areas may not be released at levels sufficient to contaminate the subsurface. Accordingly, no simultaneous fluctuations in some months and statistical significance were observed.

Warner et al. (2008) did not obtain relationships similar to those in our study between *E. coli* concentrations and the water table below ground surface. This may be because of the study’s single observation, whereas in our study, each well was monitored for about a year. We suggest that long-term surveys are important for achieving more accurate statistical results. Furthermore unlike DSG18b and DSG24, the groundwater level decreased in the wet season in DSG37. This could be because of excessive groundwater extraction from this well and other wells in the vicinity. However, because of limited scope of this study, we hope to further consider and investigate such phenomena in future.

3.5 Conclusions

Based on our long time-scale survey, we conclude that microbial concentrations in shallow groundwater in the Kathmandu Valley did not have particular spatial grouping. It is concluded that microbial concentrations in shallow groundwater were higher during wet seasons than during dry seasons, especially for dug wells. In general, we discuss two probable mechanisms for this variation: infiltration of contaminants and changes in the water level below ground surface. Increase in *E. coli* concentrations correlating with an increase in rainfall indicates a higher infiltration of contaminants during the rainy season. In addition, a moderate to strong correlation between *E. coli* concentrations and groundwater level indicate that increased groundwater levels might be responsible for the seasonal variations. Potential solutions for reducing shallow groundwater pollution would be improvements to the sewer line infrastructure and septic tanks. Since these approaches require long-term planning, we suggest point-of-use treatment of groundwater at the household level. Practical short-term and quick solutions include disinfection, filtration and boiling, particularly during the wet season.

CHAPTER 4

HEALTH RISK ASSESSMENT FROM ENTEROPATHOGENS

Health risk assessment has been highlighted by WHO as an essential tool for validation of water safety and it is also a part of risk analysis which is valuable to manage health related microbial quality of water. Although groundwater serves as important part of domestic water consumption and diarrhoea is a prevalent disease, health risk of this water source has not been assessed. This chapter presented necessity of health risk assessment, showed presence of different enteropathogens and their concentrations in shallow groundwater and estimated risk from using such water for drinking or for bathing purpose. Similarly this chapter also discussed the importance of different pathogens, consideration of different exposure pathways and incorporation of household water treatment methods in risk calculation.

4.1 Introduction

Previously human pathogens have been identified in groundwater of the valley (Haramoto et al. 2011; Tanaka et al. 2012). Exposure to pathogens through consumption of such contaminated water via drinking water, ingestion during recreation and skin contact could threaten public health (Cabelli 1983). As diarrheal cases are prevalent in developing countries (UNICEF/WHO 2009) and groundwater constitute major proportion of domestic water, groundwater use could cause high health risk.

In order to determine public health safety of water sources, United States Environmental Protection Agency (USEPA) has provided the guideline value for annual acceptable risk (10^{-4} [infection/person-year]) and the annual risk for any water sources higher than this value is considered as unacceptable. Therefore, risk assessment has been highlighted by WHO as an essential tool for validation of water safety. As risk assessment is a part of risk analysis that consisted of risk management and risk communication, it has been valuable to manage health related microbial quality of water (Haas et al. 1999). However, studies of risk assessment from groundwater are rare in Asian developing countries.

Recently, in many countries, quantitative microbial risk assessment (QMRA) has become a standard for assessing the public health risk from microbial pathogens. QMRA is specifically confined to individual pathogen and specific disease rather than indistinguishable health effects (Haas et al. 1999). In this procedure, the impact of exposure to certain pathogen will be quantified in terms of probability of infection / morbidity / mortality. It includes quantification of pathogen concentration in water source and their removal through various treatment procedures. Then they are combined with water consumption pattern and pathogen dose-response relationships, and then risk can be estimated.

An essential parameter of risk estimation using the QMRA method is the pathogen removal efficiency of water treatment method. Post-source contamination is very prevalent in developing countries, and point-of-use (POU) water treatment methods are widely practiced at the household level to safeguard against this. In order to simulate household scenario, it is necessary to incorporate POU water treatment methods into the QMRA. Groundwater has been extensively used for bathing purpose, and this is an important transmission pathway through accidental water ingestion (Pruss et al. 2002) because bathing water is usually untreated. Therefore, in addition to the risk from drinking, risk from the bathing pathway should also be estimated. Previous studies have reported less microbial contamination in tube wells compared with dug wells (Maharjan 2005; Warner et al. 2008; RDI 2008; Uy et al. 2010; Barthiban et al. 2012), and it is useful to determine whether tube well water is safer. Therefore, the risk estimation from both types of wells is necessary.

Point estimates of risk cannot convey variability of estimated risk. Therefore, probabilistic analysis should be carried out most preferably using Monte Carlo Simulations (MCS) which has recently been widely used for similar studies (Razzolini et al. 2011; Sato et al. 2013).

Hence we aimed to estimate risk of diarrhoea from enteropathogens, while using contaminated shallow groundwater, using probabilistic risk assessment and incorporating household water treatment method in risk calculation.

4.2 Method

In this study we utilized QMRA approach as described by Haas et al. (1999) to estimate risk of infection from enteropathogens in shallow groundwater of the valley. QMRA frame work consists of four steps including hazard identification, exposure assessment, dose-response relationship and risk estimation. This approach requires the quantification of the pathogens' occurrence in source water and their removal through different treatment procedures. When the pathogens' occurrence in the groundwater is combined with consumption pattern and pathogen dose-response relationships, the risk of infection can be estimated.

4.2.1 QMRA

4.2.1.1 Hazard identification

Enteropathogenic *E. coli* (EPEC), *Cryptosporidium* and *Giardia* are most prevalent enteropathogens among all that cause diarrhea in developing countries (Ochoa 2004). Among bacterial enteropathogens EPEC was most prevalent and among protozoa enteropathogens *Giardia* was most prevalent followed by *Cryptosporidium* in children (Ono et al. 2001; Uga et al. 2004; Ansari et al. 2012) as well as in adults (Pandey et al. 2002) in the valley. EPEC was the most common pathogenic *E. coli* (2/3rd of total *E. coli* strains) identified in water samples of the valley (Ono et al. 2001). Since these enteropathogens have been already identified in the shallow groundwater of the valley by several previous researches, we considered these microorganisms for detecting and estimating risk of infection from the groundwater.

4.2.1.2 Exposure assessment

According to Haas et al. (1999), the purpose of exposure assessment is to determine the number of organisms that correspond to a single exposure (dose) or the total amount or number of organisms that constitute a set of exposures. The description of exposure not only includes occurrence based on concentrations but the prevalence how often microorganisms are found or the distribution of microorganisms in space over time.

In this study we have considered two exposure pathways through which microorganisms enter human body. They were drinking pathway (groundwater used for drinking purpose) and bathing pathway (groundwater used for bathing purpose). Bathing in this study is defined as full body washing. We have included three crucial parameters in exposure assessment in this study; concentration of microorganisms in groundwater *(C)*, water ingestion rate *(V)* and removal efficiency of water treatment method *(R)*.

a. Concentration of micro-organisms (C)

As mentioned in 'hazard identification' step, we conducted risk assessment from EPEC, *Cryptosporidium* and *Giardia*.

EPEC detection:

Because EPEC, a pathogenic strain, constitutes 8% of the total *E. coli* population in water (Levine et al. 1987), the concentration of *E. coli* has been converted to that of EPEC. Details of the sampling period and microbial analysis have been discussed in *Section 2.3.1*. Briefly two seasons, dry and wet, were considered while calculating dose of EPEC in this study.

Cryptosporidium oocyst and *Giardia* cyst detection:

The procedure of detection of protozoa and the number of well sampled for this study were described in *Section 2.3.2*.

According to the protocols of the above-mentioned procedures, the value for <1 and the lower detection limit (DL) was considered 1. A small number of samples for the *E. coli* analysis and several samples for the protozoa analyses had left-censored observations, samples with concentrations below the theoretical lower DL. In order to represent such samples, half of the lower DL, 0.5, was used (Sato et al. 2013).

b. Water ingestion rate

In this study there were two types of water ingestion rates considered depending on the exposure pathways. First was voluntary water ingestion rate for drinking pathway which varies depending on dietary behaviour, cultural factors and climatic conditions. Therefore, this rate should be based on surveys in the local area. We used questionnaire survey to determine the rate which included questions about the amount of any kind of fluid, boiled water and plain water consumed per day. In drinking pathway, frequency of water ingestion has been assumed to be throughout the year but in bathing pathway the frequency per year should be surveyed. A questionnaire survey was administered to 320 individuals (age, 18–55 years) and the questions included water ingestion rate per person per day and bathing frequency. For the survey, the Kathmandu Valley was divided into three groups based on ADB (2010) according to piped water supply hours e.g. group ‘A’ (> 7 hours/ week), group ‘B’ (4-7 hours/week), group ‘C’ (< 4 hours/week). Within each group, 4 administrative units were randomly chosen and within each unit around 26 households were then randomly selected. One representative individual from each household was interviewed.

The involuntary water ingestion rate in bathing pathway was assumed to be 100 ml per bath as used by Steyn et al. (2004).

c. Removal efficiency of treatment method (R)

About 67% of households in the Kathmandu Valley used several kinds of POU water treatment methods (Shrestha et al. 2013). Ceramic water filter (CWF) is one of the most commonly used POU water treatment methods in Nepal (Low 2002; Lamichanne 2013). The effectiveness of the CWF decreases when filter unit is not regularly cleaned and flow rate decreases along with the time although hygiene is maintained (Sobsey et al. 2008). Therefore, although new CWF can have >95% removal efficiency for *E. coli* (Low 2002), CWFs used in households could have reduced efficiency. So in order to depict household scenario we assumed it to be 0.2 log (37%) for EPEC (Bielefeldt et al. 2009) and 1.58 log (97%) for *Cryptosporidium* oocysts and *Giardia* cysts (Clasen & Menon 2007). It was assumed that no treatment method was applied to bathing water. All the microorganisms were considered viable and infectious in this study.

The dose of microorganisms per exposure can be calculated by Eq. 1 as below;

$$\text{Dose } (d) = C \times V \times 10^{-R} \qquad \text{(Eq. 1)}$$

4.2.1.3 Dose–response relationship

A beta-poisson dose–response model (Eq. 2) was used for EPEC (Haas et al. 1999), whereas an exponential dose–response model (Eq. 3) was used for *Cryptosporidium* (Dupont et al. 1995) and *Giardia* (Rose et al. 1991) to compute the risk of infection/day or event (P_d).

$$P_d = 1 - [1 + d/N_{50} \times (2^{1/\alpha} - 1)]^{-\alpha} \qquad \text{(Eq. 2)}$$

$$P_d = 1 - \exp^{(-r \times d)} \quad \text{(Eq. 3)}$$

where, d = number of pathogens per exposure, N_{50} = average infecting dose (8.60×10^7), α = parameter of probability function (0.1778) (Haas et al. 1999), r = organism specific infectivity {0.01982 for *Giardia* (Rose et al. 1991) and 0.004202 for *Cryptosporidium* (Dupont et al. 1995)}.

4.2.1.4 Risk estimation

Point estimate of risk from exposure to the pathogens could be obtained by directly substituting into dose-response equation (Eq. 2 & 3) using the point estimate of means of different parameters. This method is simple to compute and convey information but it also conveys false sense of certainty in the computed number (Haas et al. 1999).

Probabilistic approaches to risk assessment take account of variability and uncertainty by using distributions rather than point estimates (Vose 2000). In the probabilistic approach all variables and parameters used in risk assessment may be regarded as distribution throughout the analysis and the final result is also given in the form of a probability distribution of given risk. In this study, we used MCS to obtain distributions of the annual probability of infection.

First, a set of random values was extracted from the distributions of the concentration of the pathogens in groundwater and water ingestion rate. The daily pathogens consumption (d) was calculated by multiplying concentration of pathogens, water ingestion rate and removal rate (Eq.1). The daily probability of infection (P_d) was obtained from the dose-response model (Eq. 2 & 3). In case of annual probability of infection (P_a) the calculation was repeated N times using Eq. 4.

$$P_a = 1 - (1 - P_d)^N \quad \text{(Eq 4)}$$

Here, N is 365 for drinking pathway and 104 for bathing pathway (from questionnaire survey). For EPEC risk estimation, N = 183 for each season (dry and wet) for drinking and 52 for each season for bathing pathway. Dry season's probability of infection ($P_{s,\ dry\ season}$) and wet season's probability of infection ($P_{s,\ wet\ season}$) were obtained from Eq. 4. And, the two seasonal probability of infection were combined to get Pa (Eq. 5).

$$P_a = 1 - (1 - P_{s,\ dry\ season}) \times (1 - P_{s,\ wet\ season}) \qquad \text{(Eq. 5)}$$

Subsequently, these equations were iterated 10,000 times to obtain the distribution of the P_a. The iteration was found to be adequate to obtain stable results. After estimating P_a (Eq. 5), we estimated combined annual risk of diarrhoea ($P_{combined}$) from all three enteropathogens from each pathway (Eq. 6). Finally, we estimated total annual risk of infection (P_{total}) from dug wells and that from tube wells by combining all enteropathogens and exposure pathways (Eq. 7). In this manuscript, the unit of risk of infection will be infection/person-year.

$$P_{combined} = 1 - (1 - P_{a,\ EPEC}) \times (1 - P_{a,\ Cryptosporidium}) \times (1 - P_{a,\ Giardia}) \qquad \text{(Eq. 6)}$$

$$P_{total} = 1 - (1 - P_{a,\ drinking}) \times (1 - P_{a,\ bathing}) \qquad \text{(Eq. 7)}$$

For each microorganism and pathway, upper limit and lower limit of the 95% Confidence Interval (CI) of risk as well as inter-quartile range were estimated. An acceptable limit of risk proposed by United States Environmental Protection Agency (USEPA), $<10^{-4}$ infections/person-year from waterborne exposure through potable water, was applied for performing risk characterisations. Here, infection is assumed to be equivalent to diarrhoea. We used median risk to describe our results. Although we could compare our results with WHO reference level of risk by estimating disease burden using disability-adjusted life years (DALYs), we lack necessary information for now and we hope and recommend assessing it in near future.

4.2.2 Sensitivity analysis

Sensitivity analysis determines the relative impact of various parameters on the computed output (Haas et al. 1999). Sensitivity analysis was performed in order to estimate the ‘relative impact’ of C, V and R on risk results according to the method described by Haas et al. (1999) and followed by Sato et al. (2013). At first, the rank correlation coefficients (Spearman rank correlation coefficient) between every parameter (C, V and R) and estimated annual risk were calculated. Then the contribution to the variance of risk was estimated by squaring the rank correlation coefficients for the parameters and normalized to 100%. The contribution to the variance is an approximated method to estimate the percentage of the variance in the risk due to each parameter (Sato et al. 2013). For risk estimation, *R* was considered as constant but in sensitivity analysis, we created minimum and maximum values of *R* by decreasing and increasing 50% to the constant value respectively and then generated 10,000 random numbers for MCS. For sensitivity analysis only, we run MCS once again with random numbers for *C*, *V* and *R* and thus the estimated risks were different from that derived from the procedure described in previous sections. Because of this limitation on the values of *R*, numerical interpretation was not performed.

4.2.3 Statistical analysis

The probability distributions of the parameters were determined and 10,000 random values of the parameters were produced by using EasyFit 5.5 Professional software. Chi-Square goodness of fit was assessed for all the data sets at the significance level of 0.05 and 0.01. The data for which Chi-Square test was not applicable they were reassessed for Anderson-Darling test at the same significance level.

Microsoft Office Excel 2007 was used to perform MCS for risk estimation. In order to compare risk between individual pathogens Independent samples t-tests were performed on risk estimated from MCS but not on the observed data. Similarly Independent samples t-test was used to compare risks between dug well and tube well. And, to compare risk between drinking pathway and bathing pathway too this test was performed. Independent samples t-test and Spearman’s rank correlation were performed using SPSS version 21.

4.3 Results

4.3.1 Detection of enteropathogens and probability distribution

On an average, 85% of dug wells and 48% of tube wells exceeded WHO guideline for drinking water (WHO 2011) for *E. coli* (0 MPN/100ml). Among dug wells, 32% and 37% were positive for *Cryptosporidium* oocysts and *Giardia* cysts respectively. Among tube wells, 7% and 13% were positive for *Cryptosporidium* oocysts and *Giardia* cysts respectively. The maximum and minimum concentrations as well as best fitted probability of enteropathogens are shown in Table 1.

Table 4.1 Descriptive statistics of enteropathogens.

Enteropathogens	**Seasons**	**Min^a**	**Max**	**Mean**	**Fitted Distribtuion**	**Goodness of fit test**
EPEC-DW (MPN/100ml)	Dry	<1	184	14	Lognormal (μ = 2.41, σ = 2.2)	Chi-square*
	Wet	<1	1589	119	Lognormal (μ = 5.05, σ = 2.6)	Chi-square*
EPEC-TW (MPN/100ml)	Dry	<1	45	1	Gamma (α = 0.04, β = 397)	Chi-square*
	Wet	<1	90	5	Lognormal (μ = 1.08, σ = 1.8)	Chi-square**
Cryptosporidium - DW (oocyst/ L)	Wet	<1	21	2	Gamma (α = 0.27, β = 8.69)	Chi-square*
Cryptosporidium - TW (oocyst/ L)	Wet	<1	22	2	Pareto (α = 4.06, β = 0.55)	Andersondarling*
Giardai - DW (cyst/L)	Wet	<1	58	6	Generalized Pareto (μ = 0.18, σ = 0.88, k=0.84)	Chi-square*
Giardia - TW (cyst/ L)	Wet	<1	22	2	Pareto (α = 2.5, β = 0.55)	Andersondarling*

a: <1 is written for no detection; DW: Dug well; TW: Tube well; μ, γ:location paremeter; σ: scale parameter; α, β, k: shape parameter; *:p-value< 0.05; ** p-value< 0.01

4.3.2 Water ingestion rate and probability distribution

Water ingestion rate best fitted with lognormal distribution (3P) ($\mu = 1.0095$; $\gamma = 1.47$; $\sigma = 0.3$) and the goodness of fit test was Chi-square (p-value < 0.05). In the questionnaire survey we asked about the amount of plain water (not boiled) and the amount of boiled water people drink per day. And, the information other types of household water treatment procedures was not asked. Almost all the respondents were drinking plain water without boiling, but we assumed that people used CWF to treat drinking water.

4.3.3 Risk of diarrhoea from dug well water

Figure 4.1 summarises estimated risks of diarrhoea from dug well water. The median risk from EPEC through the bathing pathway (0.0001 infections/person-year) contributed least to estimated risk, but it was still 10 times higher than the acceptable limit of $<10^{-4}$ infections/person-year. The median risk from *Giardia* through drinking (0.2093 infections/person-year) and through bathing (0.1911 infections/person-year) was the highest among all risks and that from EPEC through both pathways was the lowest.

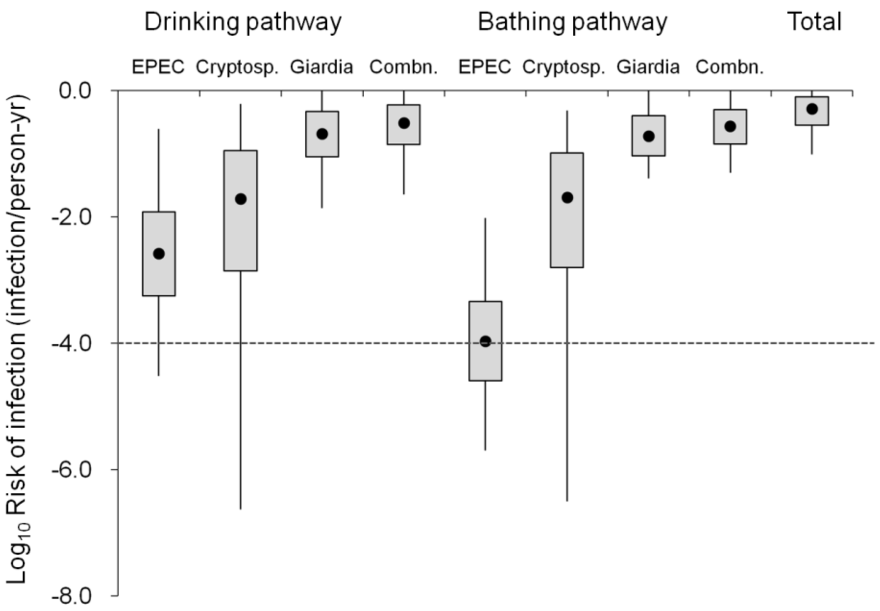


Figure 4.1 Annual risk of diarrhoea from using dug well water. Box: interquartile range; high & low lines: 95th & 5th percentiles; dot: median; Cryptosp.: *Cryptosporidium*; Combn: risk from enteropathogens combined; Total: risk from dug well; Dotted line: acceptable limit of risk.

The combined risk from all three enteropathogen was 0.3103 infections/person-year from the drinking pathway and 0.2746 infections/person-year from the bathing pathway. Because the risk from *Giardia* was much higher than that from other enteropathogens, the combined risks were dominated by that from *Giardia*. The total risk was 0.5146 infections/person-year from using dug well water, which is approximately 10^3 times higher than the acceptable limit.

4.3.4 Risk of diarrhoea from tube well water

Figure 4.2 summarises risks from tube well water. The median risk from EPEC in tube well water met the acceptable limit, from either exposure pathway but the risks from the remaining enteropathogens exceeded the acceptable limit through both exposure pathways. The highest risks were from *Giardia*; 0.1712 infections/person-year through drinking and 0.1392 infections/person-year through bathing. The combined risk from all three enteropathogens was 0.2043 infections/person-year through drinking and 0.1668 infections/person-year from bathing. Because the risk from *Giardia* was very much higher than that from other enteropathogens, the combined risks were dominated by that from *Giardia* in case of tube well water, similar to the case of dug well water. We estimated total risk from using tube well water by combining risks from all three enteropathogens through both exposure pathways. The total risk was 0.3428 infections/person-year, which exceeded the acceptable limit.

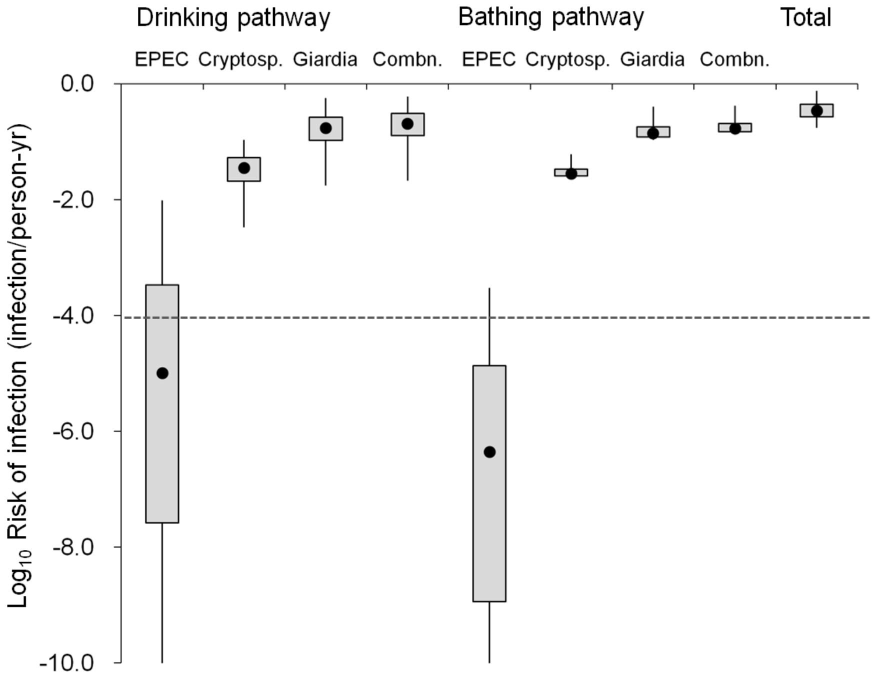


Figure 4.2 Annual risk of diarrhoea from using tube well water. Box: interquartile range; high & low lines: 95th & 5th percentiles; dot: median; Cryptosp.: *Cryptosporidium*; Combn: risk from enteropathogens combined; Total: risk from dug well; Dotted line: acceptable limit of risk.

The total risk from using dug well water and from using tube well water exceeded the acceptable limit with the order of 10^4. Hence these results indicated high public health risk while using the valley's shallow groundwater.

4.3.5 Effect of using POU water treatment parameter in risk estimation

We estimated risk with and without including POU water treatment parameter in risk calculation for drinking exposure pathway. The results are shown in Table 4.2. Upper and lower limit and 75th, 25th and median of risk distribution were presented. For all the risk statistics, except for median, 25th percentile and lower limit risks of EPEC in tube wells, risks from all enterpathogens reduced when POU water treatment parameter was included in risk estimation. Compared to EPEC, the risks from *Cryptosporidium* and *Giardia* for both well types were highly overestimated when POU water treatment parameter was ignored.

Table 4.2 Risk estimation with and without POU water treatment parameter

	Dug well					
	EPEC		*Cryptosporidium*		*Giardia*	
Risk	POU-No	POU-Yes	POU-No	POU-Yes	POU-No	POU-Yes
Upper limit	0.3632	0.2483	1.0000	0.6108	1.0000	0.9961
75th percentile	0.0191	0.0121	0.9891	0.1121	1.0000	0.4666
Median	0.0042	0.0027	0.5258	0.0194	0.9999	0.2093
25th percentile	0.0009	0.0006	0.0518	0.0014	0.9723	0.0900
Lower limit	0.0000	0.0000	0.0000	0.0000	0.4125	0.0139
	Tube well					
Upper limit	0.0153	0.0097	0.9869	0.1077	1.0000	0.5591
75th percentile	0.0005	0.0003	0.8719	0.0526	1.0000	0.2616
Median	0.0000	0.0000	0.7420	0.0350	0.9992	0.1712
25th percentile	0.0000	0.0000	0.5519	0.0209	0.9852	0.1048
Lower limit	0.0000	0.0000	0.1185	0.0033	0.4897	0.0175

POU: Point of use water treatment method; Acceptable level <0.0001 infection/ person-year

4.3.6 Sensitivity analysis

The parameters considered for sensitivity analysis were enteropathogen concentration in dry and wet seasons *(C)*, water ingestion rate *(V)* and removal efficiency of treatment method *(R)* (Table 4.3). The values for the parameters in each row represent contribution to the variance of risk from the enteropathogen in the respective row. Enteropathogen concentrations represent the largest contribution to variance of risk from EPEC in dug wells and tube wells in both dry and wet seasons. Removal efficiency represented the largest contribution to variance of risk from *Cryptosporidium* in dug wells and tube wells and that from *Giardia* in tube wells. There was little difference between the contribution of enteropathogen concentration (44%) and removal efficiency of treatment method (38%) on variance of risk from *Giardia* in dug wells. To summarise, enteropathogen concentration and removal efficiency contributed more to the variance of risk from EPEC and protozoa respectively.

Table 4.3 Contribution of parameters of risk calculation to the risk variance.

Enteropathogens	Contribution of parameters to the variance of risk (% contribution)					
	Dry season			Wet season		
	Pathogen conc. (*C*)	Water ingestion rate (*V*)	Removal efficiency (*R*)	Pathogen conc. (*C*)	Water ingestion rate (*V*)	Removal efficiency (*R*)
EPEC-DW	0.94(72)	0.29(22)	0.08(6)	0.94(73)	0.29(22)	0.07(5)
EPEC-TW	0.97(94)	0.05(5)	0.01(1)	0.99(90)	0.08(8)	0.02(2)
Cryptosporidium -DW	NA	NA	NA	0.1 (11)	0.06 (6)	0.8 (83)
Cryptosporidium -TW	NA	NA	NA	0.03 (3)	0.26 (28)	0.65 (69)
Giardia -DW	NA	NA	NA	0.41 (44)	0.16 (17)	0.35 (38)
Giardia -TW	NA	NA	NA	0.05 (5)	0.26 (28)	0.61 (67)

NA: Not available *DW: Dug well; TW: Tube well*

4.4 Discussion

This study estimated the risk of diarrhoea due to the enteropathogens, EPEC, *Cryptosporidium* and *Giardia*, from exposure to shallow groundwater in the Kathmandu Valley. The exposure pathways considered were drinking and bathing. The risks from using dug wells either for drinking or for bathing or for both purposes were 10^3 times higher than the acceptable limit. Similarly, risks from using tube well water either for drinking or for bathing or for both purposes were also 10^3 times higher than the acceptable limit. These results indicate a severe public health concern for those who are using the valley’s shallow groundwater. Hence there is an urgent need to implement risk reduction strategies. For the estimated risk from shallow groundwater to be reliable and useful for risk management in the valley or in similar settings, due consideration should be given to the following aspects;

4.4.1 Types of wells

The detection rates as well as concentrations of enteropathogens in our study were higher in dug wells than in tube wells (Table 4.1). Similar phenomena for faecal indicator bacteria were reported by Maharjan (2005) and Warner et al. (2008) in the valley, RDI (2008) and Uy et al. (2010) in Cambodia and Barthiban et al. in Sri Lanka (2012). This might indicate that tube wells have comparatively better water quality. But in our study, risks of diarrhoea from *Cryptosporidium* and *Giardia* while using tube well water were higher than the acceptable limit for both drinking and bathing purposes.

Therefore, even if tube wells have been widely reported to be less contaminated than dug wells, tube wells still represent a serious public health concern.

4.4.2 Enteropathogens

The only study that estimated risk of infection while using bore well water for drinking reported 10^{-5} infections/person-year from faecal coliforms (Emmanuel et al. 2009). This risk was similar to the risk from EPEC in our study through the same exposure pathway (10^{-5} infections/person-year). However, it was 1000 times lower than the risk from *Cryptosporidium* (0.0350 infections/person-year) and 10,000 times lower than that from *Giardia* (0.1712 infections/person-year). The risk we obtained while combining the risks from EPEC with that from *Cryptosporidium* and *Giardia* was 10,000 times higher than the risk estimated by ignoring these protozoa enteropathogens, and the combined risk exceeded the acceptable limit. Thus, excluding infective enteropathogens in the risk estimation could lead to underestimation of the potential danger. Most of the positive wells in our study had very low concentrations of *Cryptosporidium* oocysts and *Giardia* cysts, but the estimated risks exceeded the guideline value of $<10^{-4}$ infections/person-year. These results indicated that even if protozoa are detected in relatively low concentrations and in fewer samples, they could produce higher health risks because of their high virulence, infectivity and environmental resistance.

In this study, risk could be over or underestimated because we ignored pathogen infectivity, microbial die-off rate, recovery rate of microbial analysis and EPEC to *E. coli* ratio was variable and not specific to groundwater. Despite such limitations our study has uncovered important findings regarding risk related to groundwater use in the valley.

4.4.3 Conversion of *E. coli*: protozoa concentration

The mean concentration of *Cryptosporidium* in dug wells in our study was 2 oocysts/L and the risk of diarrhoea while using dug well water for drinking was 0.0194 infections/person-year. Machdar et al. (2013) reported very low risk (8.2×10^{-5} infections/person-year) from *Cryptosporidium* through an identical pathway in Ghana.

Machdar et al. (2013) estimated *Cryptosporidium* concentration (0.0038 oocysts/L) from *E. coli* concentration using an *E. coli*: protozoa ratio (10^6) derived from wastewater. Their reported concentration of *Cryptosporidium* was very much lower than that in our study. *Cryptosporidium* is highly resistant to environmental conditions than other micro-organisms such as *E. coli* (Teunis et al. 1997). So it is probable that *Cryptosporidium* concentration could be underestimated by Machdar et al. (2013). Because few protozoa enteropathogens could result into considerably higher risk of diarrhoea, faulty estimation of the concentration could misrepresent the real scenario.

4.2.4 Exposure pathways

In our study the combined risks of diarrhoea from bathing exposure were 0.2746 and 0.1668 when using dug well water and tube well water, respectively. The only study which considered the bathing pathway also estimated a similar risk from *Giardia*, 0.6760 infections/person-year (Razzolini et al. 2011) from using shallow well water. The total risk of diarrhoea from dug and tube well water increased by 67%, respectively, when the bathing exposure pathway was added in this study. With the exception of a study by Razzolini et al. (2011), all QMRA studies ignored the bathing pathway for risk estimation. There is a strong perception among people that bathing water need not to be as clean and safe as that for drinking. But our results show that there is a considerable public health risk even when using these contaminated sources for activities like bathing, when very small volumes of water could be ingested. Therefore, bathing should be considered as an important exposure pathway while doing health risk estimation studies of various water sources. Razzolini et al. (2011) used the water ingestion rate as 16ml per bathing event taking reference from Dufour (2006). While considering the rate 16ml in our study the result did not differ a lot for protozoa enteropathogens and were very much higher that the acceptable limit as defined by USEPA (Appendix 5).

4.4.5 POU water treatment method

The only two studies that estimated risk from pathogens in dug well water reported a risk of 0.9990 infections/person-year from *Giardia* (Razzolini et al. 2011) and 0.9970 infections/person-year from *E. coli* O157:H7 (Machdar et al. 2013). The respective mean concentration of *E. coli* O157:H7 was 30.4 MPN/100 mL in Machdar et al. (2013) and that of *Giardia* was 9.7 cysts/L in Razzolini et al. (2011). These mean concentrations were similar to those found in our study (EPEC = 66.5 MPN/100 mL; *Giardia* = 6 cysts/L) but the risks estimated were close to 1 infection/person-year, which was much higher than the respective risks estimated in our study (EPEC = 0.0027 and *Giardia* = 0.2093 infections/person-year). Because neither study considered the treatment method in risk estimation, risks could have been overestimated in both. In our study, when we excluded the POU water treatment method from risk estimation, the risk from *Giardia* increased from 0.2093 to 0.9999 infections/ person-year and from 0.1712 to 0.9992 infections/person-year for dug well and tube well water respectively (Table 4.2). Sato et al. (2013) considered conventional treatment methods while estimating risk from surface water in Brazil and neglected contamination of water in the distribution system and post-source contamination. In order to simulate the prevailing situation of household treatment of drinking water, the POU water treatment method should be incorporated into the risk estimation. In addition, it is recommended to consider incorporating various inexpensive POU water treatment methods in QMRA, focusing on low income households and do comparative study.

4.4.6 Sensitivity of risk estimation

Pathogen concentration (dry and wet seasons) was the major contributing parameter for risk from EPEC, whereas removal efficiency was the major contributing factor for risk from *Crytpsporidium* and *Giardia* in both types of wells. Decreasing pathogen concentrations could require long-term planning. Total risks in both types of wells were driven by protozoa enteropathogens and hence removal efficiency was the major parameter for total risk variability. We focused on the CWF and hence advising people to use this method for POU water treatment and to properly maintain the equipment could serve as a practical risk management strategy on the local level, given the present scenario.

4.5 Conclusions

Our results showed that tube wells could also pose serious risk of diarrhoea in spite of low contamination levels. When we included risks from *Giardia* and *Cryptosporidium*, the total risk increased by several thousand times. Thus, risk could be underestimated if we exclude such infective enteropathogens. In our study, the total risk of diarrhoea from shallow groundwater increased considerably when the bathing exposure pathway was included. Therefore, bathing should be considered as an important exposure pathway in addition to drinking. We estimated a very high risk of diarrhoea from shallow groundwater use, either for drinking or for bathing, which indicated a need for risk reduction strategies in the valley. We propose that household treatment should be included in risk calculations to decrease overestimation, especially in developing countries. In this study, POU treatment method appeared to have the biggest impact on risk and hence increasing CWF’s coverage and improving its efficiency could be a feasible risk management strategy on the local level in the Kathmandu Valley.

CHAPTER 5

ASSOCIATION BETWEEN DIARRHOEA OCCURRENCE AND GROUNDWATER MICROBIAL QUALITY

Groundwater as a major source among the alternatives to piped water should be examined as risk factor for diarrhoea occurrence. In this chapter the necessity of examining this association was described as well as method to analyze and results of association between diarrhoea occurrence at household level and groundwater microbial quality of water have been presented and possible explanations for the findings have been discussed. In data poor regions, interpolating groundwater microbial quality in large scale by using Geographic information system (GIS) could be an affordable solution. In this chapter we also attempted to minimize limitations of small sample size for interpolation.

5.1 Introduction

Diarrhoea is the second leading cause of healthy time lost due to illness globally (WHO 2008). Worldwide, around 760,000 children under 5 die due to diarrhea and there occur nearly 1.7 billion cases of diarrhoeal disease each year (WHO 2013). With such huge mortality and morbidity, diarrhea increases economic burden due to health cost in treatment as well as time lost at school, work, and other productive activities (Mulligan et al. 2003). The risk factors associated with diarrhoea include unsafe drinking water, lack of sanitation and poor hygiene (Pruss-Ustun and Corvalan 2006). Different socio-economic and demographic risk factors also play substantial role in diarrhoeal occurrence (Simonsen et al. 2008).

Common risk factors analyzed for association with diarrhoea included; access to piped water supply (Victoria et al. 1988; Ashraf & Yunus 1997), use of improved water sources (Helmer 1999; Shrestha et al. 2013) and access to standard amount of improved water source (Shrestha et al. 2013). In most of developing countries, utilization of piped water source lags far behind other improved water sources in developing regions (UNICEF/WHO 2012; ADB/APWF 2013). However, despite groundwater being the important water source at household level in Asia, different aspects of groundwater such as amount of use, access, purpose (bathing, laundry etc.) and quality had not been well explored as risk factors of diarrhoea unlike piped water.

In addition, although human pathogens have been already identified in groundwater (Haramoto et al. 2011; Tanaka et al. 2012), researches examining the association of groundwater microbial quality with diarrhoea were lacking. In

Bangladesh, a few studies have analyzed impact of groundwater access and depth with diarrhoea (Escamilla et al. 2011; Wu et al. 2011) because, in those areas, it was the major source of drinking water and they had no other alternatives. However, in places where piped water has been major source for drinking purpose, the impact of other major sources on diarrhoea had been still unexplored.

There had been handful of intervention studies to reduce diarrhoea through improvement in drinking water, sanitation facilities, and hygiene practices in developing countries (Fewtrell et al. 2005). According to Fewtrell et al. (2005), hygiene intervention involved education advocating specific safe behaviours, sanitation intervention includes safe excreta disposal, water supply intervention includes provision of improved water supply and water quality intervention includes removal of microbial contaminants either at source or household level. Basically water quality intervention studies have heavily emphasized drinking exposure pathway only, at household levels (Quick et al. 2002; Sobsey et al. 2008). However, drinking water is not a single exposure pathway. Water is ingested accidentally when swimming and bathing (Dufour et al. 2006) and hence, swimming in a pool and playing in interactive fountains etc. have also led to gastro-intestinal infection (Lee et al. 2002). Therefore, in case of household use of groundwater, bathing might be an important exposure pathway.

Generally in water scarce situation, less attention is paid on bathing water quality and such water is usually used untreated. Razzolini et al. (2013) estimated very high risk of diarrhea while bathing in groundwater indicating the role that this route could play on diarrheal occurrence. However, currently focus of intervention studies has yet to be shifted from drinking to bathing pathway.

Hence, we aimed to examine relationship between groundwater microbial quality and household diarrhoea occurrence while controlling for other risk factors.

5.2 Method

5.2.1 Data collection

5.2.1.1 Questionnaire survey data

We used secondary data obtained from the baseline survey of the Kathmandu Valley Water Distribution, Sewerage and Urban Development Project conducted by the Asian

Development Bank (ADB) from August to September in 2009. ADB used a multistage cluster survey; at first stage, 35 wards of five municipalities and 15 of 114 village development committees (VDCs) in the valley were selected and at the second stage, 84 geographic points were selected randomly from these wards and VDCs. A total of 20 houses were interviewed around each geographical point and 2284 total households were interviewed. Since our study focused on Kathmandu Metropolitan City (KMC) and Lalitpur Sub-Metropolitan City (LMSC), we selected 35 geographical points that were within these areas (Figure 2.4) and 942 households out of 2284 were included in this study.

No specific exclusion criteria were used for the survey but the households were excluded whose members could not be met, despite multiple visits. The protocol of this study was approved by the Ethical Review Board of University of Yamanashi.

a. Diarrheal occurrence

Diarrheal occurrence in a household is occurrence of diarrhoea in at least one family member in the past month. At each household, diarrhoea was accessed by following question; ‘Did you or anyone in your family get sick last month? If yes, what was the illness?’ The response to the question included 10 common ailments; fever, common cold, diarrhoea, dengue fever, hepatitis, typhoid, malaria, skin disease, infected wounds and other illnesses. Among the 10 ailments, response for diarrhoea and typhoid were considered as the response for diarrheal occurrence.

b. Water-related and sanitation behaviour

Water-related behaviours included sources of water used for drinking and for bathing, shallow groundwater use, POU water treatment methods (no treatment, boiling, filtration and others) and piped water storage duration. The water sources identified were piped water, shallow groundwater, stone spout, rainwater, river water, vendor’s tanker and others. For our analysis, four categories were made: using piped water only; using shallow groundwater only; using sources other than these two sources only and using mixed sources. Sanitation behaviour included types of toilets (water sealed with flush toilet, water sealed without flush toilet and pit latrine) in households.

c. *Socio-demographic characteristics*

Socio-demographic characteristics assessed were age of the household head, ethnicity (Brahmin, Chettri, Newar, Janajati or Dalit), education level of the household head (illiterate, less than secondary education and more than secondary education), household income (<USD 50, USD 50 to 150 and >USD 150) (United States Dollar, USD) and family size. In Nepal, ethnicity is a symbol of social status and ethnic minority group, Dalit, is often disadvantaged socially and economically (Baniya 2007). Ethnicity designations were based on the last names of the participants, which indicated castes in Nepal.

5.2.1.2 Groundwater microbial quality data

Primary data on shallow groundwater microbial quality were obtained by conducting field surveys and microbial analyses. Groundwater samples were collected from 36 wells (16 dug wells and 20 tube wells) in August 2009 in KMC and LMSC (Figure 2.4). *E. coli* were considered an indicator of faecal contamination in groundwater. Water samples were analysed for *E. coli* by MPN method using the Colilert reagent (IDEXX Laboratories, Westbrook, ME, USA). Details for these procedures have been explained in *Section 2.3.1*.

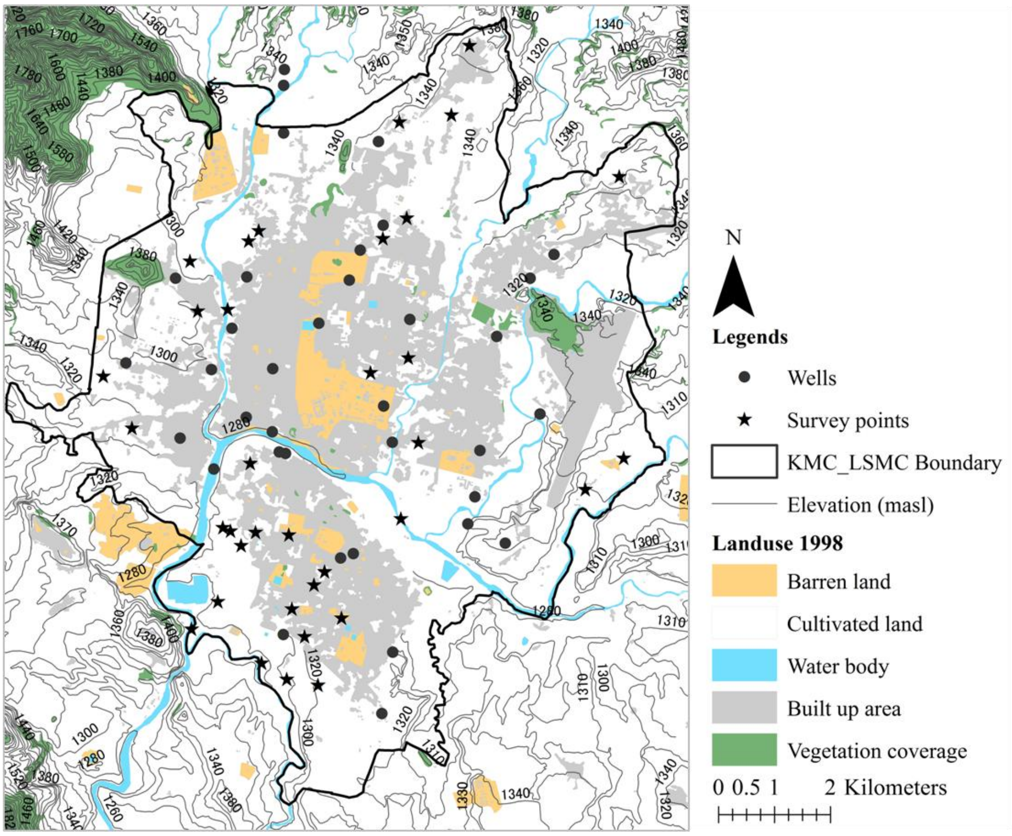


Figure 5.1 Locations of groundwater wells and questionnaire survey points

5.2.2 Analysis for relationship between groundwater microbial quality and diarrhoea occurrence

Geographical locations of groundwater sampling points (Appendix 1 & 2) and questionnaire survey locations (Appendix 3) are not similar. Therefore two approaches were used in order to merge two data sets;

5.2.2.1 Pairing of groundwater quality data with questionnaire survey locations

We paired nearby groundwater sampling points and questionnaire survey locations (Figure 5.1). A buffer (circle) of certain radius was drawn around each groundwater sampling location using ArcMap 10.1 and the closest questionnaire survey point within the circle was considered to be its pair (Figure 5.2). Four circles with radii of 0.4 k m, 0.8 km, 1.2 km and 1.6 km around groundwater sampling locations were drawn and 9, 15, 19 and 25 pairs were formed respectively and then relationship between *E. coli* concentration and percentage of households with diarrhoea occurrence was examined with the raw data set.

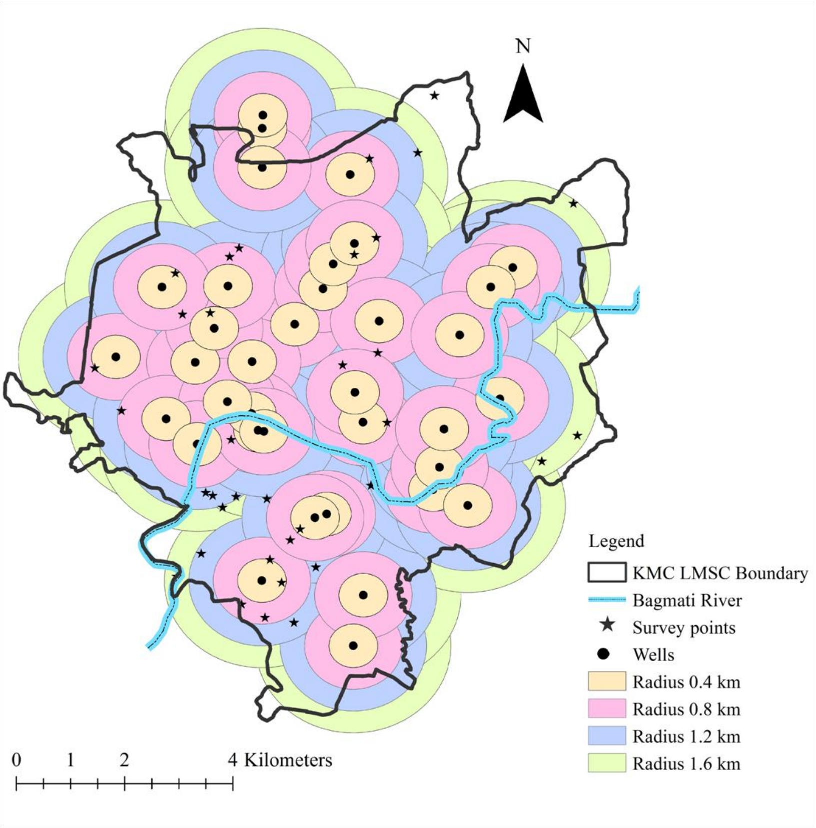


Figure5.2 Selection of pairs based on buffers of different radii

5.2.2.2 Interpolating groundwater quality data at questionnaire survey locations

Groundwater microbial quality is an environmental phenomena and understanding link between such environmental exposures and human health could be useful through Geographic Information System (GIS) (Gratell and Loytonen 1998). GIS has been used to explore link between air-pollution and health (Dunn et al. 1995; Kingham 1993) and between esophageal cancer and drought (Wu and Li 2007). In case of diarrheal disease, GIS has been widely implemented to explore spatial patterns in Thailand (Chaikaew et al. 2009) and Germany (Dangedorf et al. 2002), to evaluate health impact of improving water source in Nigeria (Njemanze et al. 1999) and to evaluate risk attributable to drinking water in Mekong watershed (Miura et al. 2007).

In this study we used GIS to interpolate groundwater microbial quality data over KMC and LSMC. Interpolation is the process of estimating the unknown data values for specific locations using known data values of other locations. In this study, we used kriging interpolation method to estimate *E. coli* concentration in the geographical locations of questionnaire survey because this method has been previously used in groundwater related studies (Kumar and Remadevi 2006; Adhikary et al. 2010;

Nas and Barktey 2010; Sakamoto et al. 2012) and has been proved to be appropriate tool.

Kriging weights the surrounding measured value to derive a prediction for each location and the weights are based not only on distance between measured locations and prediction location but also on spatial arrangement among measured points. Unlike other interpolation methods kriging investigates spatial autocorrelation of the variables. The spatial dependency is quantified using semivariogram (Burgess and Webster 1980) calculated from the measured points. It is mathematically described as the mean square variability between two neighboring points with *h* distance apart (Eq. 1),

$$\gamma(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} [z(x_i + h) - z(x_i)]^2 \qquad \text{(Eq.1)}$$

where, $\gamma(h)$ is the semivariogram as a function of the magnitude of separation distance (h) between two points, $N(h)$ is the number of observation pairs separated by h and $z(x_i)$ is the variable value at location x_i.

The empirical semivariogram is then fitted to a theoretical model such as Spherical, Exponential, Linear or Gaussian. Once the model is fitted, it will be used to calculate the spatial weight (λ) of the measured points in relation with the prediction location. Once spatial weights of all neighboring locations are calculated prediction at any point is given as the weighted sum of the measured value as shown in following equation (Eq. 2);

$$z(x_0) = \sum_{i=1}^{N} \lambda_i z(x_i) \qquad \text{(Eq.2)}$$

where, λ_i is the weight for observation $z(x_i)$ and $z(x_0)$ is the variable value at prediction location x_0.

Among many different kinds of kriging interpolation methods, ordinary kriging was chosen for this study based on the assumption of constant but unknown mean value of *E. coli* concentration in the study area. Ordinary kriging method was applied for interpolation using a geostatistical software package ArcGIS Geostatistical Analyst Extension in ArcMap 10.1. Prediction accuracy of the fitted model was determined by root mean square error (RMSE) and coefficient of determination (R^2) value obtained from cross-validation (Johnston 2004).

In this study, data of *E. coli* concentration at 36 geographical locations were available for interpolation. But as local outliers can have detrimental effect on surface prediction in kriging (Johnston 2004), we identified these outliers using ‘Exploratory Spatial Data Analysis’ and removed these from model generation, although included in surface prediction (Krivoruchko 2011). Local outliers are the measured sample points which have value within a normal range relative to all data set but have unusually high or low value relative to the surrounding. Local outliers are identified using voronoi map. Simple type of voronoi map was used for identifying the outliers. In the voronoi map the polygons which are very different from the surroundings are considered as local outliers.

The removal of local outliers in model generation improves semivariogram model and including them in surface prediction keeps the local variability intact in the spatial interpolation results. In this study, local outliers (n = 4) were identified as the using a simple voronoi map. In the simple voronoi map, a polygon surrounded by others with the values of classes separated by two other classes (symbolized by colors) should be considered as indicating a potential outlier (Johnston 2004). Then surface of *E. coli* concentration was predicted over KMC and LSMC. Then, *E. coli* concentrations at the 35 geographical locations of questionnaire survey were then extracted from the predicted surface, and these values were assigned to all households associated with these locations.

In order to examine the effect of sample size in the interpolation result we increased sample size by adding some additional points of May 2011 to data of August 2009. Ordinary kriging was performed as described in above section and *E. coli* concentrations were extracted at the 35 geographical locations of questionnaire survey. Then comparisons of this interpolation results with that obtained using 36 points were conducted.

5.2.3 Statistical analysis

a. Linear regression analysis

For the paired data set, the relationship between *E. coli* concentration and diarrhoea occurrence was examined using linear regression analysis. Coefficient of determination and the significance level of the relationship were reported. Statistical Package for the

Social Sciences version 20.0 was used for statistical analysis. Statistical significance was set at a p-value < 0.05. Scatter plot between two variables were shown for all distance pairs and both types of data sets, with and without zero values, were used.

b. Multivariable analysis

The continuous variables, shallow groundwater microbial quality, age of the household head and piped water storage duration were categorized for binary response considering their median values as cut-off points. To account for large numbers of missing values in a data set, a missing category was created for relevant variables.

To analyze difference between households that did and not did report diarrhoea during the past month within these categorical variables, we used Chi-square test. There were two levels of clustering in data, ward or VDCs level and geographical location level, which violated the independence assumption for basic regression. Thus, we used generalized estimation equation (GEE) with binary logistic model to account for the clustering effect resulting from the multistage cluster sampling design. We then assessed the association between diarrhoea occurrence and groundwater microbial quality, controlling all the potential confounders (Model 1). Other potential confounders included in this model were age, sex and education level of the household head, household income, ethnicity, piped water storage duration, POU water treatment methods, toilet types and other water sources used for drinking and bathing.

The effect of groundwater use for drinking or bathing on diarrhoea could be modified by the level of groundwater pollution. To assess possible effect modifications, we evaluated the statistical significance of a first-order cross-product term of groundwater use and microbial quality in groundwater in a model that included all the potential confounders (Model 2). Statistical significance was set at a p-value < 0.05. Statistical Package for the Social Sciences version 20.0 was used for statistical analysis.

There were missing data for age (43%), sex (40%), income (19%) and storage duration of piped water (29%). Therefore, to compare the results of multivariable analysis with and without these variables, we generated Model 3. Because there were no large changes in the results between these three models, we used Model 2 to explain the results of multivariable analysis, except for explaining the result for ‘Sanitation behavior’, because this showed a nearly significant result with this model.

5.3 Results

5.3.1 Relation between *E. coli* concentration and diarrhoea occurrence among the pairs of groundwater sampling and questionnaire survey points

The linear regression analysis was conducted between percentages of households with diarrhoea occurrence and *E. coli* concentrations in nearby points. Nine, fifteen, nineteen and twenty-five pairs made with circles of 0.4 km, 0.8 km, 1.2 km and 1.6 km radii respectively. Figure 5.3 showed the scatter plots between these two variables for pairs made with circles of different radii. The scatter plots for data excluding zero were also shown in Figure 5.3. All paired data sets showed positive relationship between *E. coli* concentration and diarrhoea occurrence. 50.4%, 35.2%, 23.1% and 18.2% variability on diarrhoea occurrence could be explained by *E. coli* concentration with the significance level < 0.05 for the pairs with 0.4 km, 0.8 km, 1.2 km and 1.6 km distance apart respectively (Figure 5.4). Coefficient of determination gradually decreased when the distance between the pairs increased (Figure 5.4) however the level of significance remained fluctuating.

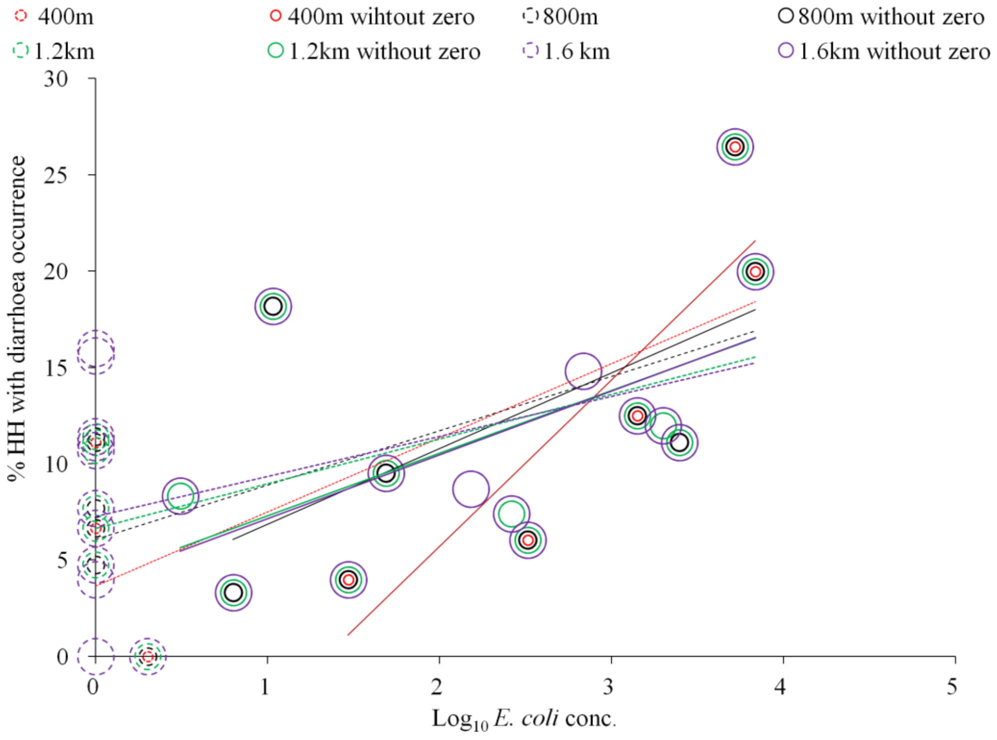


Figure 5.3 Scatter plots of *E. coli* concentration of shallow groundwater and % of households with diarhoea occurrence among pairs of groundwater sampling and questionnaire survey points.

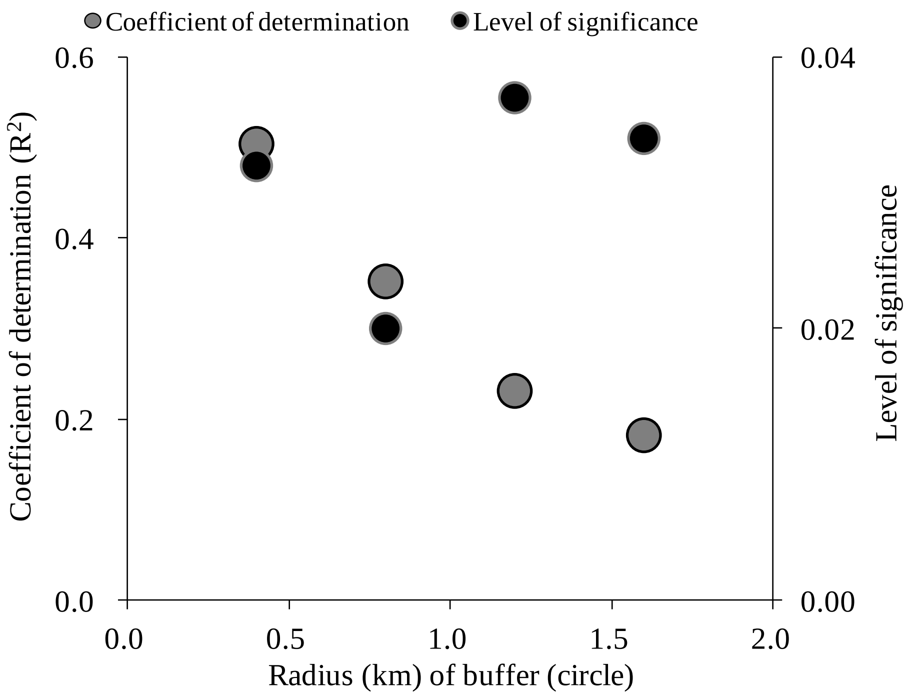


Figure 5.4 Coefficient of determination and level of significance of linear regression analysis between *E. coli* concentration of shallow groundwater and % of households with diarrhoea occurrence among pairs of groundwater sampling and questionnaire survey points

5.3.2 Interpolation of groundwater *E. coli* concentration

a. Interpolation of groundwater E. coli concentration using August 2009 data

The data for shallow groundwater *E. coli* concentration (MPN/100 ml) was not distributed normally. Thus, this data was Log_{10} transformed before conducting interpolation. Different semivariogram models were tested for model prediction; Circular, Spherical, Exponential, Gaussian, Kbessel and Stable. These models were compared basically on the basis of RMSE and R^2 (Table 5.1). Compared to all the other semivariogram models used Exponential model had lowest RMSE (1.025) and highest R^2 (0.369). The scatter plot between observed and estimated *E. coli* concentration from the values obtained from cross-validation for Exponential model was shown in Figure5. 5.

Based on the Exponential model, a surface was predicted for *E. coli* concentration which ranged Log_{10} 0.00–3.84 MPN/100 ml (Figure 5.6). The figure shows that shallow groundwater of the northeast parts had the lowest *E. coli* concentration whereas northwest and south parts had highest concentrations. After the surface was predicted, *E. coli* concentrations were extracted at the 35 geographical points of questionnaire survey. For statistical analysis, the concentration was transformed back to standard *E. coli* concentration.

Table 5.1 Error statistics and parameters of regression line for different semivariogram models

Error statistics	**Semivariogram models**					
	Circular	**Spherical**	**Exponential**	**Gaussian**	**Kbessel**	**Stable**
RMSE	1.029	1.028	1.025	1.057	1.056	1.057
Parameter of regression line						
Slope	0.328	0.329	0.346	0.298	0.299	0.298
Intercept	0.856	0.854	0.826	0.904	0.902	0.904
R^2	0.361	0.361	0.369	0.324	0.325	0.324

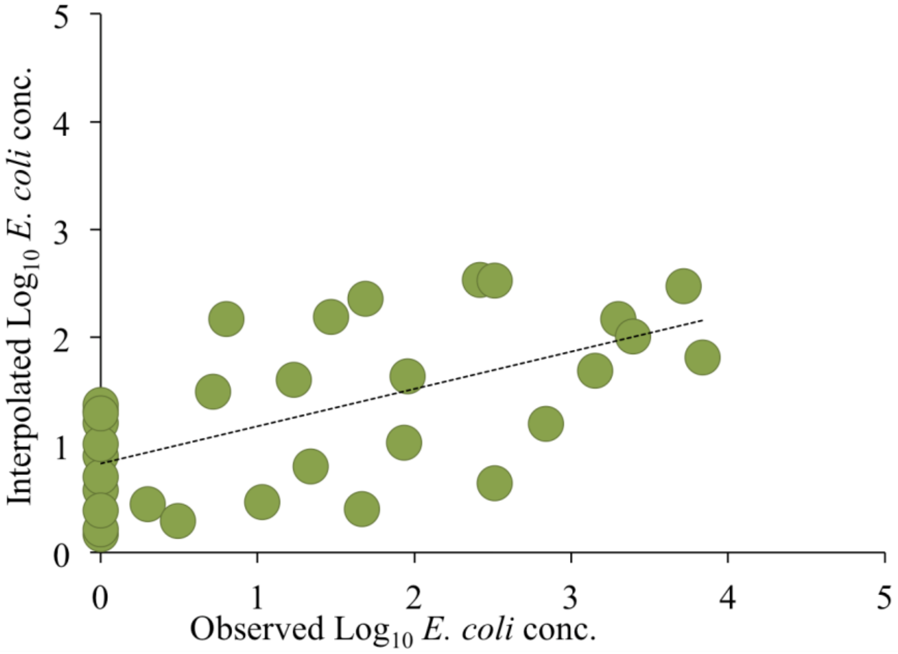


Figure 5.5 Scatter plot of observed versus interpolated *E. coli* concentration using cross-validation.

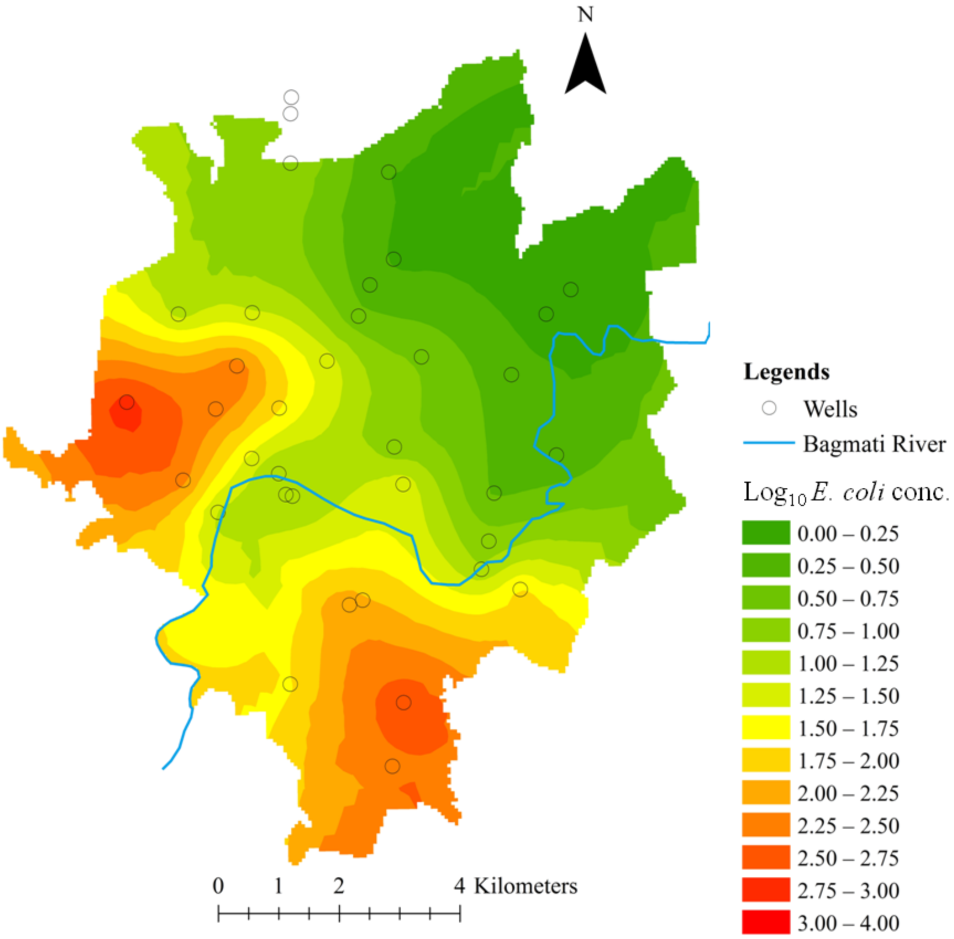


Figure 5.6 Prediction surface of Log_{10} *E. coli* concentration of groundwater over KMC and LMSC.

b. Comparison of interpolated results between 'August 2009' (N_1=36) with 'August 2009 and May 2011' (N_2=50) data

Despite the seasonal differences, some additional groundwater sampling points (n=14) were added to August 2009 data from May 2011 data and ordinary kriging was performed. As described in 'section 5.1.1.b' the procedure was followed and *E. coli* concentrations at questionnaire survey locations were predicted. The R^2 between two interpolated data was 0.8 (Figure 5.7). In addition the cross-validation results and the distribution of *E. coli* concentration in surface prediction map were also similar.

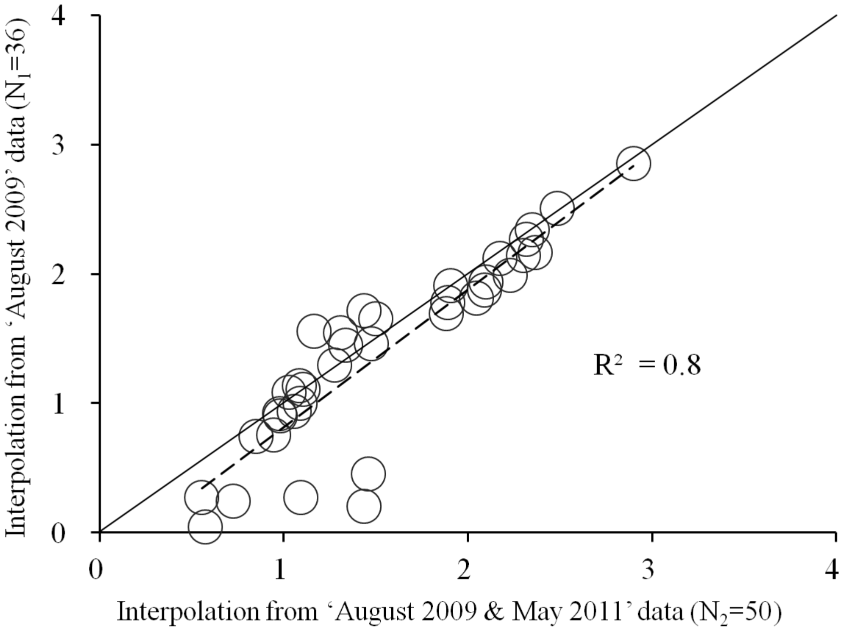


Figure 5.7 Regression between interpolated values from 'August 2009' data and 'August 2009 & May 2011' data

5.3.3 Descriptive statistics of socio-demographic variables, water-related behaviour and sanitation behaviour of households

Table 5.2 summarizes the results for different socio-demographic variables, water-related and sanitation behaviours of the households. The average age of the household head was 47 years, and the age was significantly higher for those households without diarrhoea occurrence (p-value < 0.05). Approximately 50% of the households had a male as the head of the family. The ethnic composition of our study population was as follows: 47%, Brahmin/ Chettri; 27% Newar; 24%, Janajati and 1% Dalit. Ignoring the missing data, approximately one-third of these households had an income of >USD 150 per month. The education level of household head was significantly higher among households without diarrhoea occurrence than among those with diarrhoea occurrence.

Of the 942 households surveyed, 68% used shallow groundwater for household uses. For drinking and for bathing, 6.5% and 32% of households relied only on shallow groundwater, respectively. The median *E. coli* concentration in shallow groundwater was 28 MPN/100 ml. Overall, 19% and 29% of households used more than one type of water source for drinking and bathing, respectively, and 52% and 31% of households depended only on a piped water supply for drinking and bathing, respectively. The median piped water storage duration was 24 hours. Regarding POU water treatment methods, approximately 47% of households used filtration and 23% used boiling. Surprisingly, 22% of these households did not treat water. Most of the households in this study area used water sealed toilets with almost equal proportions of with and without a flush type. By comparison, 4% of households in the capital city still relied on pit latrines.

5.3.4 Association between diarrhoea occurrence and risk factors

Of the 942 households surveyed, 87 (9.2%) had at least one family member who had suffered from diarrhoea during the past month. Table 5.3 showed the association of diarrhoea occurrence with risk factors. Groundwater use [Adjusted odds ratio (AOR) = 1.14, 95% confidence interval (CI) = 0.56–2.34; Model 1)] and level of microbial contamination of groundwater (AOR = 1.34, 95% CI = 0.85–2.13; Model 1)] were positively associated with diarrhoea occurrence. Households that used only shallow groundwater for drinking purpose had low tendency (AOR = 0.43, 95% CI = 0.08–2.17; Model 1) but that used it for bathing had high tendency (AOR = 1.42, 95% CI = 0.62–3.26; Model 1) to report diarrhoea occurrence compared with those that used only piped water.

Table 5.2 Descriptive statistics for household socio-demographic characteristics, water-related behaviour, sanitation behaviour and shallow groundwater microbial contamination with regard to diarrhoeal occurrence

Variables	Number of households, n (%) (N=942)	Number of households with diarrhoea occurrence, n (%) (N=87)	P-value (χ^2test)
Category of age [a] (years)			
Below 47	299 (31.7)	35 (11.7)	0.04
Above 47	244 (25.9)	26 (10.7)	
Missing	399 (42.4)	26 (06.5)	
Gender of household head			
Male	456 (48.4)	47 (10.3)	0.01
Female	106 (11.3)	16 (15.1)	
Missing	380 (40.3)	24 (06.3)	
Ethnicity			
Brahmin/Chettri	442 (46.9)	49 (11.1)	0.06
Newar	256 (27.2)	23 (09.0)	
Janajati	229 (24.3)	12 (05.2)	
Dalit	12 (01.3)	2 (16.7)	
Missing	3 (00.3)	1 (33.3)	
Income of household			
> USD 150	366 (38.9)	31 (08.5)	0.80
USD 50 to 150	242 (25.7)	22 (09.1)	
≤USD 50	154 (16.3)	14 (09.1)	
Missing	180 (19.1)	20 (11.1)	
Education of household head			
>Secondary level	676 (71.8)	51 (07.5)	0.03
≤Secondary level	97 (10.3)	13 (13.4)	
Illiterate	154 (16.3)	20 (13.0)	
Missing	15 (01.6)	3 (20.0)	
Family size[a]			
≤ 4	534 (56.7)	46 (08.6)	0.52
> 4	408 (43.3)	41 (10.0)	
Groundwater use			
No	305 (32.4)	24 (07.9)	0.38
Yes	637 (67.6)	63 (09.9)	
Groundwater microbial			
Low (≤ 28 MPN/ 100 ml)	446 (52.1)	36 (08.1)	0.06
High (> 28 MPN/ 100 ml)	409 (47.9)	51 (12.4)	
Sources of drinking water			
Piped water only	488 (52)	44 (09.0)	0.09
Groundwater Only	61 (06.5)	2 (03.3)	
Others only	211 (22.4)	17 (08.1)	
Mixed	180 (19.1)	24 (13.3)	

Table 5.2 Continued

Variables	Number of households, n (%) (N=942)	Number of households with diarrhoea occurrence, n (%) (N=87)	P-value (χ^2test)
Sources of bathing water			
Piped water only	295 (31.3)	21 (7.1)	0.47
Groundwater Only	298 (31.6)	31 (10.4)	
Others only	80 (08.5)	07 (8.8)	
Mixed	269 (28.6)	28 (10.4)	
Piped water storage hours[a]			
≤ 24 hrs	432 (45.9)	48 (11.1)	0.15
> 24 hrs	233 (24.7)	20 (08.6)	
Missing	277 (29.4)	19 (06.9)	
POU water treatment method			
No	202 (21.4)	16 (07.9)	0.25
Filteration	450 (47.8)	49 (10.9)	
Boiling	214 (22.7)	19 (08.9)	
Others	69 (07.3)	2 (02.9)	
Missing	7 (00.8)	1 (14.3)	
Types of toilets			
WS- flush	429 (45.6)	35 (08.2)	0.07
WS- No flush	477 (50.7)	45 (09.4)	
Pit latrine	35 (03.7)	7 (20.0)	

[a] *Mean ± Standard deviation: 46.71 ± 13.94 (Age), 4.51 ± 1.83 (Family size), 38.91 ± 26.95 (water storage*

In Model 2, the effect modification of groundwater microbial quality on the relationship between groundwater use for drinking or for bathing and diarrhoea occurrence was examined. The association of groundwater use did not change (AOR = 1.09, 95% CI = 0.54–2.20) but that of level of groundwater microbial contamination changed (AOR = 0.78, 95% CI = 0.44–1.40) with diarrhoea occurrence in households with effect modification (Model 2). Households that used highly contaminated groundwater for drinking had low tendency to report diarrhoea occurrence (AOR = 0.72, 95% CI = 0.30–17.21; Model 2) but households that used highly contaminated groundwater for bathing were significantly more likely to report diarrhoea occurrence (AOR = 5.21, 95% CI = 1.71–15.87).

For drinking purpose, households that used mixed sources (more than one type) were significantly more likely to report diarrhoea occurrence than those that used piped water only (AOR = 2.23, 95% CI = 1.20–4.15). However, for bathing purpose, this relationship was insignificant (AOR = 1.15, 95% CI = 0.56–2.36). Households that used other sources also had a higher tendency to report diarrhoea occurrence than those that used piped water (Drinking: AOR = 1.42, 95% CI = 0.62–3.30; Bathing: AOR = 1.27, 95% CI = 0.46–3.50). A longer storage duration was negatively associated with diarrhoea occurrence (AOR = 0.82, 95% CI = 0.45–1.47), whereas POU water treatment methods, filtration and boiling, were positively associated with diarrhoea occurrence compared with no treatment used (Filtration: AOR = 1.56, 95% CI = 0.72–3.37; Boiling: AOR = 1.18, 95% CI = 0.51–2.75).

Households that used pit latrines were more likely to report diarrhoea occurrence than those that had a water sealed toilet with a flush (AOR = 2.50, 95% CI =0.96–6.47), and the p value was 0.60 (Model 3). Among water sealed toilets, pour flush types were negatively associated with diarrhoea occurrence (AOR = 1.11, 95% CI = 0.67–1.84).

Compared with the Brahmin/Chettri group, the Janajati group was significantly less likely to report diarrhoea occurrence (AOR = 0.30, 95% CI = 0.15–0.61) (Model 2). Households with household heads with < secondary level of education or were illiterate were significantly more likely to report diarrhoea occurrence (< secondary level of education: AOR = 2.66, 95% CI = 1.18–5.53; Illiterate: AOR = 2.24, 95% CI = 1.12–4.48). The probability that a household reported diarrhoea occurrence during the past month could have been influenced by family size. To reduce this bias, family size was controlled for this analysis but there was not significant association with diarrhoea occurrence.

Table 5.3 Factors associated with diarrhoea occurrence at the household level

Variables	**Model 1**	**Model 2**	**Model 3**
	Adjusted odds ratio, AOR (95% Confidence Interval)		
Groundwater use			
No	1	1	1
Yes	1.14 (0.56 - 2.34)	1.09 (0.54 - 2.20)	1.02 (0.51 - 2.08)
Groundwater microbial contamination			
Low	1	1	1
High	1.34 (0.85 – 2.13)	0.78 (0.44 - 1.40)	0.78 (0.44 - 1.38)
Sources of drinking water			
Piped water only	1	1	1
Groundwater Only	0.43 (0.08 - 2.17)	0.64 (0.07 – 5.90)	0.45 (0.05 – 3.77)
Others only	1.38 (0.60 - 3.20)	1.42 (0.62 - 3.30)	1.19 (0.54 - 2.60)
Mixed	2.03(1.11 - 3.73)*	2.23 (1.20 - 4.15)*	2.10 (1.16 - 3.81)*
Sources of bathing water			
Piped water only	1	1	1
Groundwater Only	1.42 (0.62 - 3.26)	0.52 (0.18 - 1.49)	0.51 (0.18 - 1.47)
Others only	1.11 (0.40 - 3.06)	1.27 (0.46 -3.50)	1.11 (0.41 - 3.03)
Mixed	1.15 (0.56 - 2.40)	1.15 (0.56 - 2.36)	1.23 (0.60 - 2.52)
Interaction between groundwater contamination and purpose of use			
High contamination and drinking groundwater only		0.72 (0.30 – 17.21)	0.66 (0.03 – 15.19)
High contamination and bathing in groundwater only		5.21 (1.71 - 15.87)**	5.33 (1.75 -16.12)**
Piped water storage duration			
≤ 24 hrs	1	1	
> 24 hrs	0.84 (0.47 - 1.52)	0.82 (0.45 - 1.47)	
POU water treatment method			
No	1	1	1
Filtration	1.40 (0.66 - 3.00)	1.56 (0.72 - 3.37)	1.62 (0.77 - 3.40)
Boiling	1.09 (0.47 - 2.50)	1.18 (0.51 - 2.75)	1.26 (0.56 - 2.84)
Others	0.34 (0.07 - 1.62)	0.36 (0.08 - 1.70)	0.36 (0.07 - 1.75)
Category of age (years)			
Below 47	1	1	
Above 47	0.75 (0.40 - 1.43)	0.73 (0.38 - 1.41)	
Types of toilets			
WS-flush	1	1	1
WS no flush	1.16 (0.68 - 1.99)	1.15 (0.67 - 2.00)	1.11 (0.67 - 1.84)
Pit latrine	2.45 (0.88 - 6.82)	2.56 (0.91 - 7.24)	2.50 (0.96 - 6.47)
Gender of household head			
Male	1	1	
Female	1.47 (0.72 - 3.01)	1.43 (0.71 – 2.90)	

Table 5.3 Continued

Variables	Model 1	Model 2	Model 3
	Adjusted odds ratio, AOR (95% Confidence Interval)		
Ethnicity			
Brahmin/Chettri	1	1	1
Newar	0.59 (0.32 - 1.08)	0.57 (0.31 - 1.02)	0.57 (0.32 -1.02)
Janajati	0.32 (0.16 - 0.65)**	0.30 (0.15 - 0.61)***	0.32 (0.16 - 0.64)**
Dalit	0.54 (0.13 - 2.24)	0.46 (0.11 - 1.82)	0.56 (0.13 -2.35)
Income of household			
> USD 150	1	1	
USD 50 to 150	0.99 (0.50 - 1.95)	1.00 (0.51 – 1.97)	
≤USD 50	1.22 (0.61 - 2.45)	1.18 (0.57 - 2.42)	
Education of household head			
> Secondary level	1	1	1
≤ Secondary level	2.66 (1.25 - 5.65)*	2.66 (1.18 - 5.53)*	2.33 (1.11 – 4.91)*
Illiterate	2.13 (1.06 - 4.27)*	2.24 (1.12 - 4.48)*	2.11 (1.13 – 3.92)*
Sources of drinking water			
Piped water only	1	1	1
Groundwater Only	0.43 (0.08 - 2.17)	0.64 (0.07 – 5.90)	0.45 (0.05 – 3.77)
Others only	1.38 (0.60 - 3.20)	1.42 (0.62 - 3.30)	1.19 (0.54 - 2.60)
Mixed	2.03 (1.11 - 3.73)*	2.23 (1.20 - 4.15)*	2.10 (1.16 - 3.81)*
Sources of bathing water			
Piped water only	1	1	1
Groundwater Only	1.42 (0.62 - 3.26)	0.52 (0.18 - 1.49)	0.51 (0.18 - 1.47)
Others only	1.11 (0.40 - 3.06)	1.27 (0.46 -3.50)	1.11 (0.41 - 3.03)
Mixed	1.15 (0.56 - 2.40)	1.15 (0.56 - 2.36)	1.23 (0.60 - 2.52)

*Model 1: no effect modification; Model 2: effect modification; Model 3: Age, gender, income and storage duration removed; *: p-value<0.05; **: p-value<0.01; ***: p-value<0.001. WS: Water-sealed.*

5.4 Discussion

5.4.1 Relationship between *E. coli* concentration and % HH with diarrhoea occurrence for paired data sets

The result of linear regression between *E. coli* concentration and % HH with diarrhoea occurrence among pairs at certain distance apart showed positive significant association between these variables. The contribution of *E. coli* concentration on variability of diarrhoea occurrence gradually decreased with increasing distance between the pairs. It could be because as the distance increases dissimilarity between groundwater microbial quality in nearby wells also increases. If *E. coli* concentration in groundwater is spatially autocorrelated then nearby wells have similar concentration than that in distant wells and therefore, the correlation between *E. coli* concentration and diarrhoea occurrence might have been weaker in the pairs with greater distance. However, the level of significance did not decrease with distance. The pairs are not mutually exclusive for circles of different radii. For example 9 pairs are formed when taking radius 400m and the same pairs chosen with some additional new pairs when taking radius of 800m. Therefore, the common pairs for all four types of data sets might have some influence on significance level. However, there could be other reason as well which could not be explained with our results but should be analyzed in future.

For all four types of data set, *E. coli* concentration was positively and significantly associated with % HH with diarrhoea occurrence. However, there could be several other factors such as other water use behaviour, sanitation behaviour and socio-demographic factors that are simultaneously influencing diarrhoea occurrence. Therefore in order to examine independent association between the two variables multivariable analysis was carried out in this study and are discussed in following sections. Because the pairing of nearby points gave insufficient data, interpolation of groundwater microbial quality data on questionnaire survey locations was considered to be plausible method for merging two data sets and for utilizing full questionnaire survey data. Discussion on interpolation of groundwater microbial quality has been done in following section.

5.4.2 Interpolation of *E. coli* concentration

The low values are overestimated and high values are underestimated in our study which is the property of ordinary kriging interpolation method (Rezaee et al. 2011). The prediction accuracy of the kriging interpolation method using a small sample size was low in this study ($R^2 = 0.369$). However, previous studies with a small sample size (Bhowmik and Cabral 2011; Chen et al. 2012) have selected ordinary kriging as a better interpolation method than other techniques, and there might not be much difference between the error using 30 and 70 samples for ordinary kriging (Ly et al. 2011). In order to examine the changes that variation in sample size could bring to the results, we merged August 2009 with data of some additional sampling points of May 2011, despite the possibility of seasonal differences. There was good agreement between values interpolated using two data sets. The cross validation results and spatial pattern of predicted surface obtained from both data sets were similar which might somehow justify the validity of the results obtained from the 36 samples. Moreover, we could see some agreement between the observed and estimated values from interpolation from the cross-validation result, despite the limitation of the small sample size. Nevertheless, it was a factor for reducing the error (Ly et al. 2011), and for a better result, we recommend a larger sample size in future studies.

5.4.3 Association between diarrhoea occurrence and groundwater microbial quality along with other risk factors

Among all of the households surveyed, 68% used shallow groundwater for various domestic purposes; 7% and 32% relied only on it for drinking and bathing, respectively. These variations in groundwater use suggested that people preferred it less for drinking compared with for bathing and other purposes. Both groundwater use behaviour and high level of microbial contamination of groundwater were positively associated with diarrhoea occurrence, although this association was not significant. Thus, for this study, groundwater use was analyzed according to its purpose of use along with level of microbial contamination.

Households that used shallow groundwater for drinking showed a negative association with diarrhoea occurrence compared with those that used piped water. When we examined the interaction between shallow groundwater microbial quality and its use for drinking, households that used highly contaminated groundwater again showed tendency to report lower diarrhoea occurrence. Our result was indicating different story than that of Wu et al. (2011) which showed a positive association between groundwater use and diarrhoea in Bangladesh. In the Kathmandu Valley, people make water use choices based on aesthetic qualities (Warner et al. 2008). People might perceive groundwater as not fit for drinking based on its aesthetic qualities which could have resulted into very less percentage of households use it for drinking in this study (7%). Although the association did not achieve significant level, if might indicate that households drinking groundwater could have properly treated it resulting into lower risk of diarrhoea occurrence.

Almost all of the studies that investigated water quality intervention strategies for diarrhoea risk reduction focused on drinking water only (Quick et al. 2002; Sobsey et al. 2008) but rarely on bathing water. In this study, households that used piped water for bathing had a lower tendency for diarrhoea occurrence than those that used groundwater only, other alternative sources only or mixed sources (Model 1). When we examined the interaction between shallow groundwater microbial quality and its use for bathing, households that used highly contaminated groundwater for bathing were at a higher risk of diarrhoea occurrence (p-value < 0.05) (Model 2). This result indicated that poor microbial quality of shallow groundwater was a contributing risk factor for diarrhoea occurrence in this valley. Our results are in line with those of studies that reported an increased risk of gastrointestinal infections among bathers in polluted sea water (Papastergiou et al. 2012). These results emphasized the importance of bathing as an important risk factor (exposure pathway) for diarrhoea at the household level. This study focused on the effects of shallow groundwater microbial pollution on diarrhoea occurrence, but the effects of microbial quality of other water sources should also be explored for a more thorough understanding.

In this study, alternative water sources accounted for approximately 48% of drinking water consumption. Households that used alternative water sources only (p-value > 0.05) and those that used both piped and alternative sources (p-value < 0.05) were at higher risks of diarrhoea than those that used piped water only. Shrestha et al. (2013) reported similar results on domestic water use in this valley. As people choose water based on its aesthetic qualities, the quality of sources other than piped water could have been overestimated and treated inadequately.

The POU water treatment method has been commonly and successfully studied for diarrhoea risk reduction at the household level (Quick et al. 2002; Sobsey et al. 2008). Although the association between diarrhoea occurrence and POU water treatment methods was expected to be negative, our study results showed an opposite tendency. However, in this study, households using boiling and filtering treatment methods showed a higher tendency of diarrhoea occurrence than those using untreated water. Shrestha et al. (2013) also reported lower risk of diarrhoea among households which did not treat water. In this study the questionnaire was not structured so as to include the factors that influence effectiveness of the POU treatment method i.e. maintenance of filter (Sobsey et al. 2008), inadequate boiling, storage condition of boiled water etc.

Therefore we could not further explore such result. But for future studies we highly recommend to include them.

Apart from water-related behaviour, sanitation is an important and widely studied factor used in efforts to reduce diarrhoea. In this study, households with pit latrines were at a higher risk of diarrhoea occurrence than those with water sealed toilets with a flush. Our result is in line with that of Moraes et al. (2003) who reported more frequent diarrhoea episodes among children in households with pit latrines than among children in households with flush toilets. Other studies also found significant associations between unimproved latrines and diarrhoea (Semba et al. 2011). Although the association between pit latrines and diarrhoea and the relevant explanations are not new in the public health field, this finding is important because despite the tremendous efforts made for sanitation interventions, pit latrines remain in use in the capital city of this country and have a significant health impact.

In this study, education level of the household head was significantly negatively associated with diarrhoea occurrence. This finding is in line with that of Shrestha et al. (2013). This negative association between education levels with health can be explained by attaining a healthy life style and by more work and better socio-economic status (Ross & Wu et al. 1995).

Our findings should be interpreted in the light of some limitations. First, diarrhoea occurrence was self-reported. Therefore, caution should be taken when interpreting the findings of this study. Because the data were self-reported, there is a possibility of over- or underreporting. However, minimization of bias was attempted by using trained interviewers and by assuring the confidentiality of the participants' information. Second, the prediction accuracy of the kriging interpolation method using a small sample size was low in this study ($R^2 = 0.369$). Despite such limitations, this study has uncovered important findings regarding groundwater use, groundwater microbial quality and diarrhoea occurrence at the household level in the Kathmandu Valley.

5.5 Conclusions

Based on the results of our study, we conclude that shallow groundwater microbial pollution is an important risk factor for causing diarrhoea in households in the

Kathmandu Valley. Bathing in groundwater with high levels of microbial contamination is considered to be a key exposure pathway for diarrhoea occurrence and underscores the need to pay more attention to the quality of water being used for purposes other than drinking. The water quality of sources perceived as secure by households could play a role in causing diarrhoea through the drinking pathway in this valley. Moreover, our study results underscore the need for reducing pit latrine use to reduce the risk of diarrhoea in households. Because the education level has a protective effect against diarrhoea occurrence, diarrhoea risk reduction interventions should focus on awareness raising campaigns that emphasize on households that have less well-educated household heads. In future studies, the associations of the microbial quality of different water sources such as piped supply, bottled water and stored water, and the associations of different possible exposure pathways with diarrhoea occurrence should be examined to garner deeper insights into potential risk factors.

CHAPTER 6

SUMMARY OF RESEARCH

6.1 Conclusions

Major proportion of the household water in developing countries has been fulfilled by sources other than piped water and among these different alternatives groundwater is the most popular source. However, in many countries microbial pollution of groundwater especially in urban areas is common which a serious public health concern. Our study has analyzed the aspect of spatial and seasonal variation of the microbial quality, assessed the risk of diarrhoea from different enteropathogens that contaminated groundwater and finally explored the association between diarrhoea occurrence and groundwater microbial quality stratified by the purpose of use at the household level. These three main objectives of this study have summarized the following important conclusions:

1. Microbial concentrations in groundwater of neighboring wells in the Kathmandu Valley were different and there were apparently no spatial clustering or groupings. Absence of particular relationship between *E. coli* concentration and wastewater loading provided us no clue about possible mechanism of such spatial variation. In this study different time periods showed that *E. coli* and total coliform concentrations in both dug and tube wells were higher in wet season compared to that in dry. Therefore our long time scale survey revealed that seasonal variation of microbial quality existed in shallow groundwater of the valley with wet season being poorer in quality. Seasonal variation of rainfall was coherent with that in dug and tube well microbial concentration. So, one possible mechanism of seasonal variation could be infiltration of more contaminants in wet season. Likewise, in monitoring wells pattern of monthly fluctuations in *E. coli* concentrations were similar to that of water level below ground surface. Therefore, another possible mechanism of seasonal variation could be increase in water level below ground surface in wet season.

2. Groundwater of the valley found to be contaminated with enteropathogens; EPEC, *Cryptosporidium* oocysts and *Giardia* cysts. We estimated high risk of diarrhoea from using the groundwater either for drinking or bathing purpose especially from *Giardia* and *Cryptosporidium*. While the acceptable level of risk should be below 0.0001 infection/ person-year, in our study the combined risks of diarrhoea from

bathing exposure were 0.2746 and 0.1668 when using dug well water and tube well water, respectively. It is concluded from the results that bathing should be considered as an important exposure pathway of infection. In our study, risk of diarrhoea from *Cryptosporidium* and *Giardia* while using tube well water were higher than the acceptable limit through drinking as well as bathing pathway. Therefore, even if tube wells have been widely reported to be less contaminated than dug wells, they still pose a serious public health concern. In our study, when we excluded the POU water treatment method from risk estimation, the risks from different enteropathogens were overestimated; risk from *Giardia* increased from 0.2093 to 0.9999 and from 0.1712 to 0.9992 infections/person-year for dug well and tube well water respectively. Therefore regarding methodological consideration, especially for developing country setting, ignoring household water treatment method could lead to overestimation of risks. In this study, POU treatment method appeared to have the biggest impact on estimated risk compared to concentration of microorganisms and water ingestion rate.

3. Results of interpolations using 36 samples were similar with what obtained using 50 samples but with these small sample sizes prediction accuracy seemed to be low. In multivariable analysis, bathing in highly contaminated groundwater showed higher risk of diarrhoea occurrence in households of the valley, while other confounding variables were controlled. Hence this result also showed that bathing in groundwater seemed to be a key exposure pathway for diarrhoea occurrence. Although insignificant, households which use groundwater were positively associated with diarrhoea occurrence. Households which used more than one type of water sources (mixed sources) for drinking were significantly at higher risk of diarrhoea occurrence. Likewise, those household with illiterate or having education below secondary level were also at higher risk of diarrhoea occurrence compared to the households with well educated household head. The use of pit latrine instead of water sealed toilets in households was also positively associated with diarrhoea occurrence and this association was nearly significant.

6.2 Generalization of the results

The results of this study may not be applicable to every developing country. We focused our research on urban area of a developing country which is suffering from water scarcity and groundwater is serving as major household water source. Therefore, our findings are specifically representative of the urban areas of developing countries which are depending on shallow groundwater to mitigate water problem and which have socio-economic and cultural similarities. The results could be representative of urban areas of the neighbouring countries of Nepal, such as India, Bangladesh, which have socio-economic and cultural similarities. The data collection period in Chapter 5 was wet season. Hence, the results, particularly from Chapter 5, were further limited to the rainy season of the year.

6.3 Contributions

1. For the first time in the valley, this long time scale survey has established that the quality of groundwater diminishes in wet season. This seasonal prospect will be helpful in setting a priority for implementation of pollution control strategies in resource poor settings such as in developing countries. Further, this study proposed two possible mechanisms of seasonal variation which reflected that potential solutions could be improvements to the sewer line infrastructure and septic tanks. But these approaches require more time and resources. Therefore local and feasible strategies such as introducing household water treatment methods could be short-term solutions particularly during the wet season.

2. Health risk assessment of shallow groundwater in terms of diarrhoea from enteropathogenic microorganism contributed in revealing the potential health effect of the current level of microbial pollution this water source. This study emphasized the importance of consideration of *Cryptosporidium* and *Giardia* in risk assessment although their concentrations in water sources are very low because they are highly infectious and are very resistant to environment and treatment. This study highlighted inclusion of POU water treatment method as methodological correction of ‘dose estimation’ step adapted particularly for developing countries where such practice is common phenomena. Not only estimation of risk this study also highlighted factors which could be underscored more in order to reduce health risk. In the valley POU

treatment method had biggest impact on risk estimation and hence increasing CWF's coverage and improving its efficiency could be a feasible risk management strategy on the local level in the Kathmandu Valley.

3. A methodology was developed to integrate GIS with epidemiological data. This type of integration could be helpful for researches in data poor regions, especially in developing countries. This study highlighted the contribution of contaminated groundwater in diarrhoea occurrence of household and it is possibly the first study that examined different prospects of groundwater use and its relationship with diarrhoea. The key findings underscored the contribution of contaminated groundwater and bathing pathway in diarrhoea occurrence and underscored the need to pay more attention to alternative water sources besides piped water and to the quality of water being used for purposes besides drinking. It also suggested the need of reducing use of pit latrines and of emphasizing households with less well-educated household heads for awareness raising campaigns.

6.4 Recommendation for future researches

In this study different aspects of groundwater microbial quality were analyzed and key findings were identified and interpretations were done. However, this study had several limitations which could be improved in future researches. In addition, the findings of this study have recommended further explorations on some additional aspects. So some such recommendations are listed as follows;

1. We would like to recommend exploring more on spatial variability of microbial quality of groundwater which could be further helpful in developing pollution control strategies for particular areas. Other mechanisms of seasonal variation of microbial concentration such as influence of rivers should be properly analyzed.

2. Regarding risk assessment, we would like to recommend for risk assessment through other exposure pathways. In our study, different parameters of risk estimation were not included such as pathogen infectivity, microbial die-off rate, recovery rate of microbial analysis etc. This might have caused over or under-estimation of risk. Therefore, it would be better if future studies could

incorporate these parameters. We used only CWF as POU water treatment method, however, there are many other treatment methods such as boiling, SODIS, chemical etc. which could be more effective and inexpensive as wells. Therefore, we would recommend incorporating various inexpensive POU water treatment methods in QMRA, focusing on low income households and do comparison. As POU water treatment method had largest impact on risk estimation among different parameters, it could be useful to conduct small scale intervention study using POU water treatment method at the local level.

3. In our study, small sample size for interpolation of groundwater microbial quality could have affected the prediction accuracy. Therefore, we would like to recommend interpolation studies with larger sample size for reducing error and obtaining better result.

4. Our study analyzed microbial quality of groundwater only even though other water sources were also included in questionnaire survey. Therefore in future it is highly advisable to analyze microbial quality of other major water sources and conduct multivariable analysis. In this study many parameters were ignored such as maintenance of CWF, method of boiling and water storage condition post boiling, water storage hygiene in multivariable analysis. We would like to recommend for inclusion of such factors in questionnaire survey in future studies.

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Appendices

Appendix 1

Geographic coordinates of dug wells used for groundwater sampling in this study

Location ID	Well type	Location	N60	E60	Elevation (masl)
DSG01	Dug well	Radhavawan	27°41'34.7"	85°18'44.1"	1295
DSG04	Dug well	Gongabu	27°44'25.1"	85°18'50.4"	1309
DSG06	Dug well	Khumaltaar	27°39'16.1"	85°19'44.9"	1326
DSG14	Dug well	Chuchepati	27°43'1.9"	85°21'20.4"	1337
DSG17	Dug well	Bijuli Bazar	27°41'29.6"	85°19'50.7"	1295
DSG18	Dug well	Tahachal	27°42'8.6"	85°17'22.8"	1304
DSG24	Dug well	Kamaladi Ganesthan	27°42'28.1"	85°19'9.9"	1305
DSG26	Dug well	Vimsenthan	27°42'5.3"	85°18'10.3"	1281
DSG27	Dug well	Kupandole	27°41'24.8"	85°18'47.9"	1278
DSG29	Dug well	Patan Campus	27°40'34.8"	85°19'29"	1326
DSG31	Dug well	Voldhoka	27°39'46.3"	85°19'50.9"	1292
DSG32	Dug well	Afaldole	27°39'55"	85°18'50.2"	1319
DSG34	Dug well	Sankata	27°42'5.8"	85°18'44.3"	1313
DSG35	Dug well	Tamogalli	27°42'25.7"	85°18'21.7"	1307
DSG36	Dug well	Kuleshowr	27°41'31.6"	85°17'52.9"	1280
DSG37	Dug well	Nukabahal	27°40'32.6"	85°19'22"	1308
DSG40	Dug well	Koteshowr	27°40'49.4"	85°20'32.6"	1306
DSG41	Dug well	Patan	27°40'15.4"	85°19'8.39"	1330
DSG48	Dug well	Teaching Hospital	27°44'8.12"	85°19'48.35"	1342
DSG49	Dug well	Maharajgunj	27°44'22.83"	85°20'13.67"	1354
DSG50	Dug well	Gongabu	27°43'52.84"	85°18'45.83"	1304
DSG51	Dug well	Newbuspark	27°44'7.12"	85°18'38.81"	1310
DSG52	Dug well	Gairidhara	27°43'4.59"	85°19'41.54"	1318
DSG53	Dug well	Naxal	27°42'48.35"	85°19'37.68"	1319
DSG54	Dug well	Gyaneshowr	27°42'30.62"	85°19'54.90"	1318

Appendix 2

Geographic coordinates of tube wells used for groundwater sampling in this study

Location ID	**Well type**	**Location**	**N60**	**E60**	**Elevation (masl)**
TSG01	Tube well	Radhavawan	27°41'34.7"	85°18'44.1"	1295
TSG03	Tube well	Manamaiju	27°44'32.9"	85°18'50.8"	1319
TSG05	Tube well	Teku	27°41'41.9"	85°18'29.6"	1287
TSG07	Tube well	Koteshowr	27°40'39.9"	85°20'53.4"	1300
TSG08	Tube well	Tinkune	27°41'2.7"	85°20'36.5"	1287
TSG10	Tube well	Gaurighat	27°42'50.3"	85°21'7.2"	1346
TSG11	Tube well	Chhetrapati	27°42'50.9"	85°18'29.9"	1305
TSG12	Tube well	Maharajgunj	27°43'57.5"	85°19'43"	1341
TSG13	Tube well	Naagpokhari	27°42'49.3"	85°19'26.8"	1308
TSG15	Tube well	Sinamangal	27°41'43.5"	85°21'12.7"	1312
TSG16	Tube well	Shantinagar	27°41'25.5"	85°20'39.3"	1295
TSG19	Tube well	Gairidhara	27°43'4.1"	85°19'32.9"	1298
TSG20	Tube well	Bhatbhateni	27°43'16.3"	85°19'45.6"	1300
TSG22	Tube well	Gyaneshowr chowk	27°42'30"	85°20'0.4"	1300
TSG23	Tube well	Anamnagaar	27°41'47.4"	85°19'45.9"	1306
TSG25	Tube well	Sovavagwati	27°42'50.3"	85°17'50.4"	1305
TSG28	Tube well	Sanepa, Lalitpur	27°41'16.4"	85°18'11.6"	1279
TSG30	Tube well	Samakhusi	27°44'1.7"	85°18'50.4"	1305
TSG33	Tube well	Kupandole	27°41'24.1"	85°18'51.4"	1285
TSG39	Tube well	Gaushala	27°42'21.6"	85°20'48.6"	1291
TSG42	Tube well	Tapatali bridge	27°41'20.05"	85°18'58.11"	1288
TSG43	Tube well	Kupandole	27°41'18.31"	85°18'58.71"	1294
TSG44	Tube well	Chakupat	27°41'9.22"	85°19'19.24"	1289
TSG45	Tube well	Chyasal	27°40'32.05"	85°19'51.67"	1300
TSG46	Tube well	Chyasal	27°40'32.05"	85°19'51.67"	1300
TSG47	Tube well	Juweagal	27°41'6.44"	85°19'13.42"	1296
TSG55	Tube well	Manamaiju	27°44'28.43"	85°18'53.18"	1311

Appendix 3

Geographic coordinates of questionnaire survey sites selected by ADB

Cluster	Municipality	Ward no.	N60	E60
1	Kathmandu	2	27°43'9.93"	85°19'45.49"
2	Kathmandu	4	27°44'10.73"	85°20'23.48"
3	Kathmandu	6	27°43'19.98"	85°19'58.72"
4	Kathmandu	6	27°43'40.42"	85°21'56.63"
5	Kathmandu	10	27°41'29.56"	85°20'5.14"
6	Kathmandu	13	27°42'2.22"	85°17'10.24"
7	Kathmandu	14	27°41'36.69"	85°17'26.17"
8	Kathmandu	15	27°42'34.29"	85°18'2.66"
9	Kathmandu	15	27°42'58.81"	85°17'58.38"
10	Kathmandu	16	27°43'13.81"	85°18'36.55"
11	Kathmandu	16	27°43'8.70"	85°18'30.89"
12	Kathmandu	18	27°42'35.09"	85°18'19.28"
14	Kathmandu	32	27°42'11.26"	85°19'59.55"
15	Kathmandu	32	27°42'3.97"	85°19'38.55"
16	Kathmandu	34	27°40'52.08"	85°19'55.45"
18	Kathmandu	35	27°41'21.93"	85°21'59.08"
19	Kathmandu	35	27°41'6.53"	85°21'37.76"
47	Lalitpur	2	27°41'19.37"	85°18'31.82"
48	Lalitpur	2	27°40'47.77"	85°18'16.58"
49	Lalitpur	3	27°40'44.14"	85°18'53.22"
50	Lalitpur	3	27°40'45.44"	85°18'34.76"
51	Lalitpur	3	27°40'46.08"	85°18'20.82"
52	Lalitpur	3	27°40'38.95"	85°18'26.73"
53	Lalitpur	4	27°40'11.37"	85°18'13.87"
54	Lalitpur	4	27°39'57.78"	85°17'59.16"
57	Lalitpur	5	27°40'7.73"	85°18'54.97"
58	Lalitpur	5	27°39'54.11"	85°19'2.00"
60	Lalitpur	13	27°39'41.11"	85°18'38.11"
61	Lalitpur	14	27°39'33.13"	85°18'52.06"
62	Lalitpur	14	27°39'30.22"	85°19'9.44"
66	Lalitpur	19	27°40'3.25"	85°19'22.51"
67	Lalitpur	20	27°40'19.50"	85°19'7.21"
68	Lalitpur	20	27°40'26.02"	85°19'13.11"
81	Kathmandu	3	27°44'44.89"	85°20'33.52"
85	Kathmandu	3	27°44'7.22"	85°19'54.57"

Appendix 4

Questions for water ingestion rate and bathing frequency

1. How much water (in amount e.g. 2L, 1L, 1.5L) you drink each day including drinking beverages? ....

2. How much water (in amount e.g. 2L, 1L, 1.5L) you drink without boiling each day ? ....

3. How much boiled water (in amount e.g. 2L, 1L, 1.5L) you drink each day?...............

4. How many times do you take bath per week? …………………………..

Appendix 5

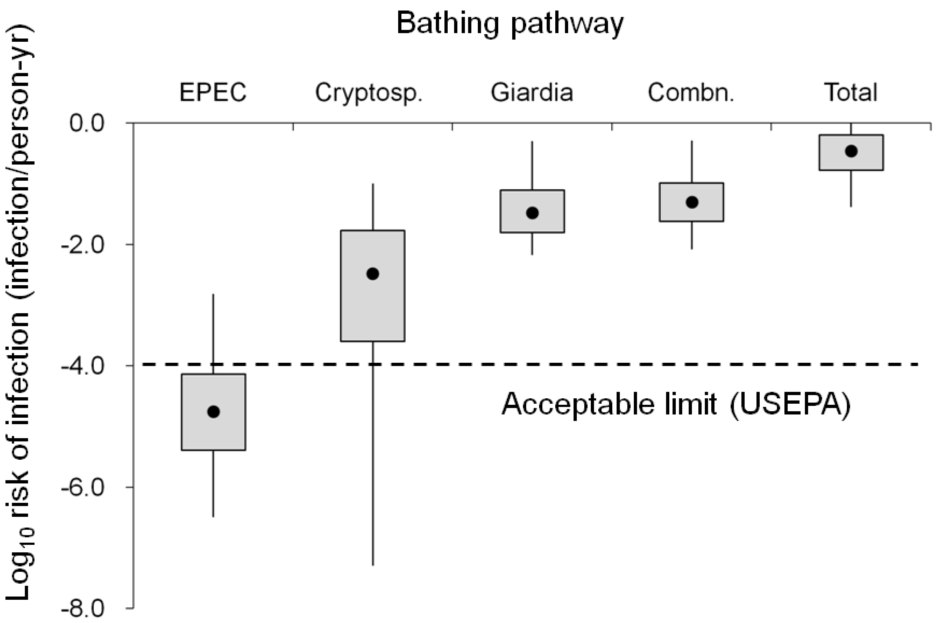

Figure Annual risk of diarrhoea from using dug well water. Box: interquartile range; high & low lines: 95th & 5th percentiles; dot: median; Cryptosp.: *Cryptosporidium*; Combn: risk from enteropathogens combined; Total: risk from dug well; Dotted line: acceptable limit of risk.

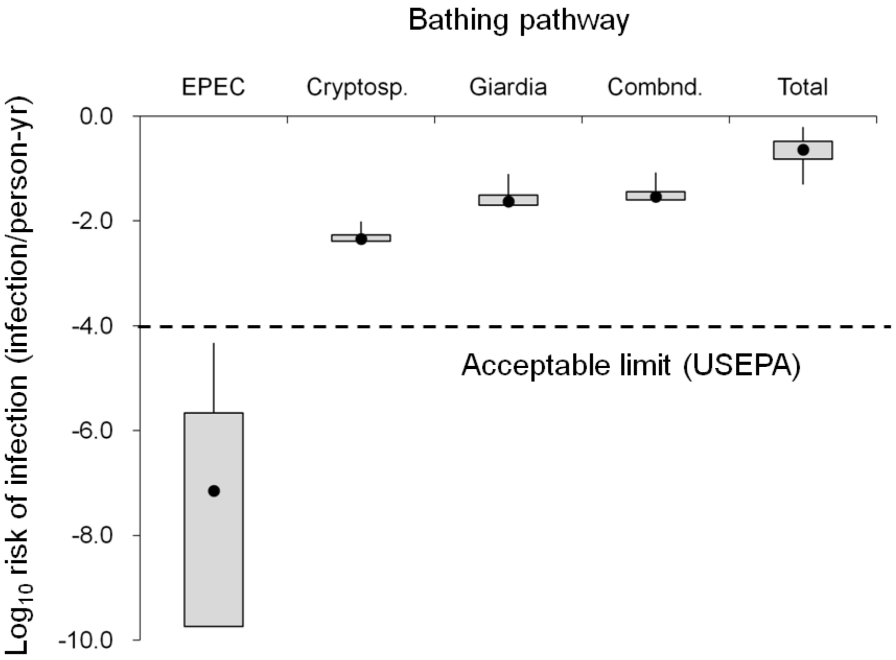

Figure Annual risk of diarrhoea from using tube well water. Box: interquartile range; high & low lines: 95th & 5th percentiles; dot: median; Cryptosp.: *Cryptosporidium*; Combn: risk from enteropathogens combined; Total: risk from dug well; Dotted line: acceptable limit of risk.