A large-scale study of bacterial contamination of drinking water and its public health impact in Nepal

SK Rai,[1,2,3] K Ono,[4] J-I Yanagida,[5] S Ishiyama-Imura,[5] M Kurokawa[6] and CK Rai[1]

[1]Shi-Gan Int'l College of Science & Technology (SICOST), [2]Nat'l Institute of Tropical Medicine & Public Health Research, and [3]Dashimura Foundation, Kathmandu, Nepal; [4]Departments of Public Health and [5]Microbiology, Kobe Tokiwa University and [6]Kobe Institute of Health, Kobe, Japan

Corresponding author: Prof. Dr. Shiba Kumar Rai' Shi-Gan Int'l College of Science & Technology (SICOST), Kathmandu, Nepal; e-mail: drshibakrai@gmail.com

ABSTRACT

Bacterial contamination of drinking water is a major public health in Nepal. A large scale study on contamination of drinking water was done covering all three ecological belts (mountain, hill and *Terai*) in all five (eastern, central, western, mid-western and far-western) development regions of Nepal during 2009-2011. Of the total 506 water samples studied, one-forth (25.1%; 127/506) were visually turbid. Bacteriologically, 88.5% (448/506) samples were positive for total coliform (TC) whereas 56.5% (286/506) were positive for fecal coliform (FC) (*Esch. coli*). The TC positive rate ranged from 53.8% in Damak (Jhapa) to 100.0% in different districts. The FC positive rate varied more widely ranging from 10.0% in Bharatpur City (Chitawan) to 100.0% in Baglung Township (Baglung) with over 50.0% in most of the districts (over 75.0% in eight districts). Both TC and FC positive rate were highest in Far-western Development Region (DR). High TC positive rate (96.7%) in Far-western DR was followed by Western DR (93.9%), Eastern DR (89.2%), Central DR (87.0%) and Mid-western DR (74.6%). Highest FC positive rate (65.5%) in Far-western DR was followed by Med-western DR (63.5%), Western DR (55.9%), Central DR (53.2%) and Eastern DR (52.0%). TC positive was highest (90.7%) in hills followed by mountain (89.7%) and *Terai* (plain) (84.1%) belt. In contrast, FC positive rate was highest (66.2%) in mountain, followed by hills (58.0%) and *Terai* (49.7%). Of the total 506, 335 were piped tap water, 129 were boring water, 16 natural tap (spout), 16 were well (sallow/deep well) and 10 were mineral/uroguard treated water. TC positive rate was very high (81.2% to 100.0%) in different type water samples (piped tap: 90.1%; boring water: 85.2%; natural spout/tap: 81.2%; well water 100.0% and mineral water/uroguard treated water: 80.0%). FC positive rate ranged from 0.0% in mineral water/uroguard treated water to 93.7% in well water samples. These findings are of serious public health concern with regard to both endemicity and outbreak of waterborne diseases in the country.

Keywords: Large-scale study, drinking water contamination, public health impact, Nepal.

INTRODUCTION

Contamination of drinking water is one of the great public health problems worldwide particularly in developing countries. Safe drinking water supplies improves people's health which, in turn, improve their productivity and livelihoods (earns more, eats more nutritious foods and enjoys healthy lives). MDG Target-10 under the MDG Goal-7 calls for the world to halve, by 2015, the proportion of people without sustainable access to safe drinking water and basic sanitation.[1] The MDGs and associated targets prioritize safe drinking water on the basis of its ability to reduce poverty and contribute to significant socioeconomic and environmental gains.

Consumption of drinking-water contaminated with human and animal feces is the major cause of various water-borne diseases (diarrhea, dysentery, typhoid fever, hepatitis and others) caused by various pathogenic bacteria, viruses and parasites. Three and half million people die every year due to unsafe water and lack of proper sanitation.[2] Diarrhea alone causes four percent of all deaths and five percent of health loss to disability, mostly among children in developing countries.[3] About two thirds of the world's populations underserved by water live in Asia.[4] One third of Asians do not have access to safe and sustainable water supply. Even worse, one half do not have access to improved sanitation. Many countries in Asia including Nepal reportedly have increased the drinking water supply coverage but there is great problem in both water safety and sustainability.[4-14]

According to "WHO guidelines for drinking water quality", *Escherichia coli* (fecal coliform bacilli) must not be detectable in any 100 ml sample water of (1) all water directly intended for drinking, (2) treated water entering the distribution system, and (3) treated water in the distribution system; and outbreak control measure and investigative action (increase in the concentration of free chlorine to greater than 0.5 mg/litre throughout the system and drinking of boiled water) must be taken immediately if they are detected.[15] However, drinking water quality in most of the developing countries is not achieved/maintained.

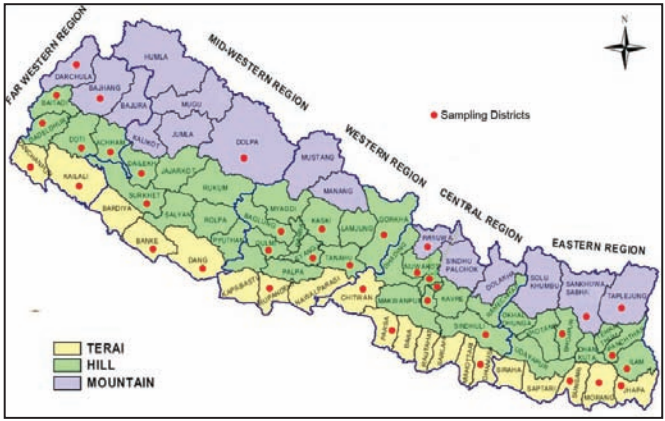

Fig. 1: Map of Nepal showing water sampling districts (marked with red dot)

The reported data from Nepal have showed high rate of drinking water contamination[6-14] as well as soil.[16,17] This has resulted into the endemicity and/or frequent outbreak of various waterborne diseases (mainly diarrhea) including cholera in the country.[7,18,19] Until recently, the killer diarrheal diseases significantly occupied the list of "top-ten diseases"[7,10,,11,13,18,19] and constituted one of the major public health problems in Nepal. According of demographic health survey 2011, two-third of hospital outpatient department visiting patients presents with water and sanitation related diseases.[20]

Twelve percent of children suffer from diarrhea.[21] However, no large-scale study on drinking water contamination has been carried out till date. In this paper, we report the status of drinking water contamination in different 41 cities/ townships/ VDC areas in 37 districts across the country covering mountain, hill and *Terai* regions in Eastern, Central, Western and Mid-western and Far-western Developmental Regions.

MATERIALS AND METHODS

This is the largest study ever conducted on bacterial contamination of drinking water. A total of 506 water samples collected from 41 cities/townships/VDC areas in 37 districts [Eastern Development Region (DR): *Taplejung, Panchthar, Ilam, Jhapa, Morang, Sunsari, Sankhuwasabha* and *Bhojpur;* Central DR: *Sindhuli, Dhanusha, Parsa, Kathmandu, Lalitpur, Bhaktapur, Chitawan, Nuwakot* and *Rasuwa;* Western DR: *Gorkha, Tanahun, Kaski, Syangja, Baglung, Rupandehi* and *Gulmi;* Mid-western DR: *Banke, Dolpa, Surkhet, Dailekh* and *Dang,* and Far-western DR: *Kailali, Kanchanpur, Achham, Doti, Baitadi, Dadeldhura, Darchula* and *Bajhang*] across the

Fig. 2: Locals using sallow well (1), sampling from piped tap (2) using commercially available water test system. Out of five samples (tubes) four are positive for TC (3) and out of those four TC positive samples three (showing apple green color) are FC (*Esch. coli*) bacilli positive (4)

country (Fig. 1). Ecologically, 6 districts (Sample n = 68) were from Mountain Region, while 20 and 11 districts were from Hill (Sample n = 281) and *Terai* (Plain) (Sample n = 157) Regions, respectively. The water samples were collected in the year 2009 and 2011. Samples were collected directly from piped tap water, well (sallow well and deep well) and deep bored tube wells being used by the locals as drinking water source. In some places, samples well collected from household reservoir tanks and taps.

The water samples were tested for total coliform (TC) as well as for fecal coliform (*Escherichia coli*, the accurate indicator of fecal contamination) using commercially available test system called Colilert (marketed by IDEXX Laboratory, Tokyo, Japan). The test system consists of tubes containing dehydrated (dry powder) media with two indicators, namely, ONPG (orthonitrophenylgalactoside) that gives yellow color upon the growth of TC bacilli whereas another indicator the MUG (4-methylumbelliferyl-B-D-glucuronide) gives bluish fluorescence under 365 nm of UV light in a dark environment upon the growth of FC (*Esch. coli*). Water sampling and testing was done as described by the manufacturer. Briefly, water was filled in the tube leaving about 1.0 to 1.5 cm space at the top of the tube, screw capped and mixed well to dissolve the powder (dehydrated media). The tubes were then incubated at 37^{0}C for overnight. In mountain and hill districts where incubator was not available, the water filled tubes were wrapped in vinyl bags and were placed in the groin area during sleeping (incubation at about 35^{0}C). The tubes showing yellow color after incubation were regarded as "TC positive" and the tubes showing blue fluorescence under 365nm of UV light were regarded as "FC (*Esch. coli*) positive" (Fig. 2).

RESULTS

Of the total 506 water samples studied, one-forth (25.1%; 127/506) were visually turbid. Bacteriologically, 88.5% (448/506) samples were positive for total coliform (TC) whereas 56.5% (286/506) were positive for fecal coliform (FC) (*Esch. coli*) (Table-1). The range of TC positive rate ranged from 53.8% in Damak (Jhapa District) to 100.0% in different districts. The FC positive rate varied more widely ranging from 10.0% in Bharatpur City (Chitawan District) to 100.0% in Baglung Township (Baglung District). However, in most of the districts the FC positive rate was over 50.0% (with over 75.0% FC positive rate in eight districts) indicating that the drinking water being used by the locals is not safe for drinking and is of great public health concern.

TC positive rate was highest (96.7%; 58/60) in Far-western DR. This was followed by Western DR (93.9%; 119/127), Eastern DR (89.2%; 91/102), Central DR (87.0%; 134/154) and Mid-western DR (74.6%; 47/63) (Fig.-3; Table-1). FC positive rate was also highest in Far-western DR (65.5%; 40/60). However, it was interesting to note that the FC positive rate decreased with west to eastern direction; 63.5% (40/63) in Mid-western DR, 55.9% (71/127) in Western DR, 53.2% (82/154) in Central DR and 52.0% (53/102) in Eastern DR. Both TC and FC positive rates were highest in Far-western DR compared to other four DRs.

TC contamination was highest (90.7%; 255/281) in hilly region followed by mountain region (89.7%; 61/68) and *Terai* (plain) region (84.1%; 132/157) (Fig.-4). In contrast, FC positive rate was highest (66.2%; 45/68) in mountain region, followed by hilly (58.0%; 163/281) and *Terai* regions (49.7%; 78/157). FC positive rate in *Terai* was significantly low compared with mountain and hilly region.

Of the total 506 water samples, 335 were piped tap, 129 were boring water, 16 natural tap (spout), 16 were well (sallow/deep well) and 10 were mineral/Uroguard treated water (Table-2). TC positive rate was very high (81.2% to 100.0%) in different type water samples (piped tap: 90.1%; boring water: 85.2%; natural spout/tap: 81.2%; well water 100.0% and mineral water/uroguard treated water: 80.0%). FC positive rate ranged from 0.0% to 93.7% (piped tap: 60.6%; boring water: 42.6%; natural spout/tap: 81.2%; well water 93.7% and mineral water/uroguard treated water: 0.0%).

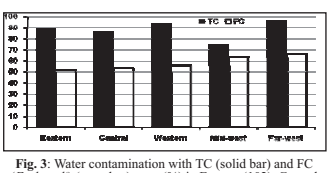

Fig. 3: Water contamination with TC (solid bar) and FC (*Esch. coli*) (open bar) rates (%) in Eastern (102), Central (n=154), Western (n=127) and Mid-western (n=63) & Far-western (n=60) DRs

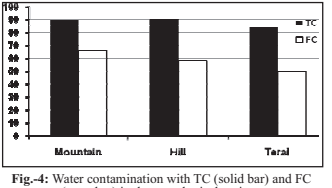

Fig.-4: Water contamination with TC (solid bar) and FC (open bar) in three ecological regions (mountain, hill and plain) of Nepal

Table-1: TC and FC (Esch. coli) positives rates in 41 cities/townships/VDC areas in 37 districts of all five DRs, Nepal

Region	District	Sampling place	Total n	Total Coliform		Fecal Coliform	
				+ve n	%	+ve n	%
Eastern Devt Region Total n = 102 TC: 89.2% FC: 52.0%	Taplejung	Fumling Township	11	11	100.0	7	63.6
	Sankhuwasabha*	Khandbari Township	11	11	100.0	9	81.8
	Panchthar	Fithim Township	11	11	100.0	8	72.7
	Ilam	Ilam Township	10	10	100.0	5	50.0
	Bhojpur	Bhojpur Township***	10	8	80.0	6	60.0
	Jhapa	Damak City	13	7	53.8	2	15.4
	Morang	Biratnagar City	15	13	86.7	8	53.3
	Sunsari	Daran City	11	10	90.9	4	36.4
		Itahari City	10	10	100.0	4	40.0
Central Devt Region Total n =154 TC: 87.0% FC: 53.2%	Rasuwa*	Dhunche Township	12	12	100.0	9	75.0
	Nuwakot	Trisuli Township	10	10	100.0	8	80.0
	Sindhuli	Kamalamai Township	30	29	96.6	17	56.6
	Kathmandu	Kathamndu City***	28	26	92.8	15	53.6
	Bhaktapur	Bhaktapur City***	8	6	75.0	5	62.5
	Lalitpur	Patan City***	15	13	86.6	9	60.0
	Chitawan	Bharatpur City	10	6	60.0	1	10.0
		Mugling Township	11	11	100.0	7	63.4
	Parsa	Birgunj City	15	10	66.6	5	33.3
	Dhanusha	Janakpur City	15	11	73.3	6	40.0
Western Devt Region Total n =127 TC: 93.9% FC: 55.9%	Gorkha	Aruchanaute area	10	9	90.0	5	50.0
	Tanahun	Damauli Township***	13	13	100.0	10	76.9
	Kaski	Lethnath City	14	14	100.0	8	57.1
		Pokhara City	21	18	85.7	8	38.1
	Syangja	Syangja Township	18	17	94.4	13	72.2
	Baglung	Baglung Township	10	10	100.0	10	100.0
	Gulmi	Turang VDC	8	8	100.0	2	25.0
	Rupandehi	Butawal City	15	13	86.7	8	53.3
		Bhairahawa City***	18	17	94.4	7	38.8
Mid-Western Devt Region Total n = 63 TC: 74.6% FC: 63.5%	Dolpa*	Dunai & Jufal Township***	20	13	65.0	13	65.0
	Dailekh	Surkhet-Jumla road side	8	5	62.5	3	37.5
	Surkhet	Birendranagar City	11	7	63.6	6	54.5
	Banke	Nepalgunj City	14	14	100.0	11	78.6
	Dang	Lamahi area	10	8	80.0	7	70.0
Far-Western Devt Region Total n =60 TC: 96.7% FC: 66.7%	Darchula**	Gokuleswor & Chamelia	7	7	100.0	3	42.8
	Bajhang**	Chainpur & Bagthala***	7	7	100.0	4	57.1
	Baitadi**	Satbanj & Patan areas	6	6	100.0	3	50.0
	Dadeldhura**	Dadeldhura township	6	6	100.0	4	66.7
	Achham**	Magalsen & Sanfe Bagar	7	7	100.0	5	71.4
	Doti**	Dipayal***	6	6	100.0	5	83.3
	Kailali**	Dhangadi & Attariya	9	8	88.8	6	66.6
	Kanchanpur**	Mahendranagar City	12	11	91.7	10	83.3
Total			506	448	88.5	286	56.5

*Rai *et al*, Ref. No. - 10; **Rai *et al*, Ref. No. - 13; ***and adjacent areas

DISCUSSION

Contamination of drinking water is one of the major public health problems (due to both morbidity and mortality associated with various kinds of waterborne diseases) worldwide particularly in least developed countries including Nepal.[22] It is resulted due mainly to poor sanitation. Of the various waterborne diseases, diarrheal disease alone amounts for 4.1% of total DALY (disability adjusted life years) global burden of disease and is responsible for the deaths of 1.8 million people every year.[3] In Nepal, diarrhea alone is estimated to be associated with yearly death of 30,000 and morbidity of 3.3 episodes per child.[23] Therefore, the problems of unsafe drinking water, poor sanitation and improper hygiene has also been considered as "silent emergency". As the safe drinking water supplies has immediate impact in improving people's health and their productivity and thereby in their livelihoods, the MDG Target-10 under the MDG Goal-7 calls for the world to halve, by 2015, the proportion of people without sustainable access to safe drinking water and basic sanitation.[1]

Nepal has given high priority in drinking water supply and sanitation[24-26] and progress has been made significantly but with significant disparities in rural and urban areas, ecological belts and DRs, and ethnic groups particularly in the case of sanitation.[25] To promote the sustainable and cost effective demand-led rural water supply and sanitation services with community ownership Rural Water Supply and Sanitation Fund Development Board has been constituted.[26] A total of 80% of the population have access to drinking water and 43% of the populations have access to sanitation.[5] However, there is big issue of sustainability and in many places particularly in rural areas, the piped water taps are non-serving. According to statistics available,[5] of the 38,307 drinking water projects/schemes so far completed only 17.9% have been operating in good condition while 38.8% require some maintenance, 11.8% require huge repair, 21.0% require improvement and extension, 9.1% need to be reconstructed and remaining 1.3% are not in a position of being brought back to operation. Even in the case of serving piped water taps, there is question of safety (quality) as has been shown by available reports.[6-13]

The available reports on bacterial contamination of drinking water are of small-scale study. We therefore, conducted this large-scale study covering all three ecological regions [mountain, hill and plain (*Terai*)] areas in all five DRs using a new water testing system (Colilert, marketed by IDEXX Laboratory, Tokyo, Japan).

This study showed very high TC positive rate (88.5%). This was in agreement with the previous studies from Nepal,[6-14] however, the water testing method was different (previous studies employed the conventional MPN method). Further, some of the previous studies have been limited only to TC contamination. In this study, we used commercially available new test system mentioned above. Importantly, this study revealed that 56.5% of total water samples tested was contaminated with FC (*Esch. coli*). This was much higher than reported earlier (25.6%)[14] and correlated well with the poor sanitary condition in the country[4,5,20,24,27] and some kind of disorder in the water supply system[5,10,13] and/or no continuous supply of water. Recently, though few places in the country have been declared as open defecation free (ODF) areas (district as a whole or Village Development Committee areas), around 57% of population still practice open defecation because they do not have the toilet and 59% of public and community schools have no toilet.[25,27] Furthermore, contamination of piped water is attributed to intermittent supply of water. During the stoppage of supply, sewage water from the sewage pipe laid parallel or crossing each other and/or from the surroundings environment with poor sanitation enters into the drinking water pipe with physical disorder even in the big cities including capital city.[5-8,28] Furthermore, heavy source contamination, improper treatment of water before distribution and poor hygienic/sanitary practice also contribute in the contamination of drinking water. As a result, outbreaks of diarrheal diseases including

Table-2: TC and FC (Esch. coli) positive rates in water samples collected from different sources

No	Water sampling source	Total n	Total coliform		Fecal coliform	
			+ve n	%	+ve n	%
1	Piped tap	335	302	90.1	203	60.6
2	Boring water*	129	110	85.2	55	42.6
3	Natural tap (spout)	16	13	81.2	13	81.2
4	Well (sallow/deep well*) water	16	16	100.0	15	93.7
5	Mineral water/uroguard treated water	10	8	80.0	0	0.0
	Total	**506**	**448**	**88.5**	286	56.5

*Boring water and deep well samples were mostly from *Terai* (plain) areas.

cholera occur even in the capital city-Kathmandu (and other two big in the valley)[19,29] which is considered to be low risk district for diarrhea outbreak.[22]

Recently, even the bottled mineral water of different brands in the market has been reported to be contaminated with FC.[12] According to the report, 91.2% and 59.6% of bottled mineral water were contaminated with TC and FC, respectively. This was in agreement with the findings of mineral water factory monitoring done by the authority recently. Monitoring revealed that mineral water factories had poor and unacceptable quality maintenance and many were sealed off by respective authority. This very fact poses a big challenge to the government's commitment in ensuring the access to safe drinking water and sanitation for all by 2017[24] and it is appealed to the people's representatives, politicians, policy-makers and government officials to act on this issue.[30]

High positive rate of both TC and FC in Far-western DR[13] appeared to be attributed to low overall developmental indicators in the region compared with other DRs. Of the five development regions, Far-western DR has lowest sanitation coverage (47.3%).[25] The lowest TC positive rate seen in Mid-western DR was still very high (74.6%) whereas the lowest FC positive rate was 52.0% (in Eastern DR). High FC positive rate observed in Far-western and Mid-western DRs correlated well with the report on sanitary coverage[25] and frequent outbreaks and high number of deaths due to waterborne diseases (particularly the diarrhea) in both of these two DRs.[22]

Despite the high sanitary coverage,[25] TC contamination rate was highest in hilly region followed by mountain and *Terai* (plain) regions, respectively. This must be attributed mainly to source contamination due to overflow of the sewage in sloppy topography. On the other hand, in spite of very low sanitary coverage[25] (49%) in *Terai*, the TC positive rate was low in *Terai* belt. However, present finding was still very high (84.1%) compared with previous findings of household water contamination study (61%).[9] Open defecation is still common in *Terai* belt and the *Terai Dalit* community has lowest sanitation coverage.[25] However, FC positive rate seen in this study was significantly lower than in other two ecological belts. In contrast, FC positive rate was highest in mountain region, followed by hilly and *Terai* belts. The reason why FC positive rate in *Terai* was significantly lower compared with positive rates in mountain and hilly belts might be due to the use of "deep boring" water. Most of the deep boring water samples in this study were negative for bacterial contamination.

All open sallow well water samples were positive for TC whereas 93.7% were FC positive. This appeared to be due to its open and unprotected condition. At many places, fecal/sewage materials enter into the sallow wells. As the covering of water vessels alone reduce both FC and TC counts in stored water by 50%[31] wells must be protected by covering them. On the contrary, however, closed piped water (TC: 90.1% and FC: 60.6%), deep boring water (TC: 85.2% and FC: 42.6%), natural spout/tap water (both TC and FC: 81.2%) and mineral water/uroguard treated water (TC: 80.0%) were also heavily contaminated. In this study, none of the mineral water and uroguard treated water was positive for FC. Interestingly, this was in contrast to the findings of Subedi and Aryal (59.6% FC positive rate).[12] One reason could be the difference in the test system as well as batch of bottled mineral water (Subedi and Aryal's study was conducted before the recent inspection/monitoring of mineral water factories done by the concerned authority).

The number of days per year lost due to waterborne diseases in Nepal, reportedly, was higher (10 days) for TC-positive households compared with TC-negative households with "double cost" of the medical expenditure.[9] Study done in nearby country Bangladesh has shown reduction in diarrheal diseases in children in rural areas by environmental and behavioral modifications.[32] Keeping in view of the importance of sanitation a campaign for declaration of ODF districts and/or VDCs is launched. However, sporadic open defecation has been occurring because of no public toilets in terrain of farming villages and/or no change in behavior of locals. On the other hand, locals in the villages do not boil water regularly despite awareness about the risks associated with drinking of unpurified water.[33] Furthermore, water boiling at home alone does not confer protection as the people on many occasions become compelled to drink raw/untreated water outside.

Present findings well correlated with existing sanitary condition[5,22] and very high prevalence of the waterborne diseases,[7,20-22] which are of great public health concern. This has serious negative impact not only on people's health but also on their productivity and thereby in their livelihoods (economic concern). These findings, clearly indicated an urgent need of taking steps for water safety such as effective water treatment at the treatment plant, maintenance of physical integrity of distribution system, as well as introduction of a simple, safer and cost-effective water treatment system at household/community level, improvement of sanitary and hygienic condition, point-of-use quality monitoring and continuous surveillance, and involvement of the local people and concerned stake holders in these actions which, in turn, immediately improve the health, productivity and livelihood of the local people contributing in achieving MDGs as has been targetted.[1]

ACKNOWLEDGEMENTS

Water samples were collected during the visits (research studies and/or monitoring visits) to the districts included. Mr. Anadna Karki (Lecturer), Ms. Reena Mukhiya (Instructor) and Ms. Amita Prajapati (MSc Microbiology student) did sampling in Sindhuli District while Mr. Jitendra Shrestha, Mr. Deependra Gautam, and Rajendra Prasad Joshi (MSc Microbiology students) sampled in Gorkha, Gulmi and Kailali Districts, respectively while Mr. Suraj Adhikari and Ms. Shikha Gautam (MSc Microbiology students) collected additional samples from Rupandehi District. Mr. Rabin Tamrakar did the graphic work sampling site on the map of Nepal.

REFERENCES

1. UN Millennium Development Goal. http://www.unmillenniumproject.org/goals/gti.htm#goal7
2. Water.org. http://water.org/learn-about-the-water-crisis/billion/
3. WHO. http://www.who.int/water_sanitation_health/diseases/
4. ADB/UNDP/UN ESCAP/WHO. Asia water watch 2015 – Are countries in Asia on track to meet target 10 of the Millennium Development Goals? 2005.
5. DWSS (Department of Water Supply & Sewerage). Business Plan for 2010-2013 (2067/68-2069/70). Ministry of Physical Planning and Works, Govt of Nepal 2011.
6. Adhikari RK, Rai SK, Pokharel BM, Khadka JB. Bacterial study of drinking water of Kathmandu, Neal. *J Inst Med (Nepal)* 1986; 8: 313-6.
7. Ono K, Rai SK, Chikahira M *et al*. Seasonal distribution of enteropathogens detected from diarrheal stool and water samples collected in Kathmandu, Nepal. *Southeast Asian J Trop Med Public Health* 2001; 32: 520-6.
8. Ghimire G, Pant J, Rai SK, Choudhary DR, Adhikari N. Bacteriological analysis of water of Kathmandu Valley. *J Nepal Assoc Med Lab Sci* 2007; 8: 45-7.
9. Atreya K, Panthee S, Sharma P. Bacterial contamination of drinking water and the economic burden of illnesses for the Nepalese households. *Int'l J Environ Health Res* 2006; 16:385-90.
10. Rai SK, Ono K, Yanagida J-I, Kurokawa M, Rai CK. Contamination of drinking water in mountainous region in Nepal. *Nepal Med Coll J* 2009; 11: 281-3.
11. Ishiyama S, Yanagida J-I, Rai SK *et al*. Study on microbial enteropathogenic contamination of water supply in Kaski District, Nepal. *Bull Kobe Tokiwa University* 2011; 3: 29-33 (*Text in Japanese*).
12. Subedi M, Aryal M. Public perception about drinking jar water and its bacteriological analysis. *Nepal Med Coll J* 2010; 12: 110-4.
13. Rai SK, Ono K, Yanagida JI, Ishiyama S, Rai CK. Bacterial contamination of drinking water in Far-western Development Region, Nepal. *J Univ Grants Commission (Nepal)* 2012; 1: 68-73.
14. Prasai T, Lekhak B, Joshi DR, Baral MP. Microbiological analysis of drinking water of Kathmandu Valley. *Scientific World* 2007; 5: 112-4.
15. WHO. Guidelines for drinking-water quality, 3rd edition (incorporating first & second addenda), Vol. 1 - Recommendations, Geneva 2008.
16. Rai SK, Uga S, Ono K, Rai G, Matsumura T. Contamination of soil with helminth parasite eggs in Nepal. *Southeast Asian J Trop Med Public Health* 2000; 31: 388-93.
17. Shrestha A, Rai SK, Basnyat SR, Rai CK, Shakya B. Soil-transmitted helminthiasis in Kathmandu, Nepal. *Nepal Med Coll J* 2007; 9: 166-9.
18. Rai SK, Rai G, Hirai K, Abe A, Ohno Y. The health system in Nepal-an Introduction. *Envt'l Health Preventive Med* 2001; 6: 1-8.
19. Ise T, Pokhrel BM, Rawal S *et al*. Outbreak of cholera in Kathmandu Valley, Nepal. *J Inst Med* (Nepal) 1994; 16: 196-201.
20. NDHS (Nepal Demographic Health Survey 2011), Ministry of Health & Population 2012.
21. MoHP (Ministry of Health and Population). http://www.mohp.gov.np/english/about_moh/fact_sheet.php
22. EDCD (Epidemiology and Disease Control Division), Department of Health Services, Nepal 2010.
23. Pokhrel D, Viraraghavan T. Diarrhoeal diseases in Nepal vis-à-vis water supply and sanitation status. *J Water Health* 2004; 2: 71-81.
24. GoN (Government of Nepal), National Water Plan 2005.
25. GoN (Government of Nepal), MDGs Acceleration Framework: Improving Access to Sanitation 2012.
26. RWSSFDB (Rural Water Supply & Sanitation Fund Development Board); http://www.rwss.org
27. Nepal MDG Progress Report 2010.
28. Rai SK, Shrestha HG, Nakanishi M *et al*. Hookworm infection recorded at an University Teaching Hospital in Kathmandu, Nepal over one decade period. *Japanese J Trop Med Hyg* 1997; 25: 81-4.
29. Karki A, Tiwari BR. Prevalence of acute diarrhoea in Kathmandu Valley. *J Nepal Med Assoc* 2007; 46: 175-9.
30. WaterAid Nepal, 2011; www.wateraid.org/nepal
31. Wright J, Gundry S, Conroy R. Household drinking water in developing countries: a systematic review of microbiological contamination between source and point-of-use. *Trop Med Int'l Health* 2004; 9: 106-117.
32. Aziz KM, Hoque BA, Hasan KZ *et al*. Reduction in diarrhoeal diseases in children in rural Bangladesh by environmental and behavioural modifications. *Trans Roy Soc Trop Med Hyg* 1990; 84: 433-8.
33. Kovalsky AN, Lacey SE, Kaphle UR, Vaughn JM. Risk perception and water purification practices for water-borne parasitic infections in remote Nepal. *Trop Doct* 2008; 38: 229-31.