

Assessment of Bacterial Load in Tube Wells, Filling Stations and Drinking Water Sources of Livestock and Poultry in Jammu

Najimaana Wani¹, Asif Iqbal^{2*}, Manzoor Ahmad³ and S.K. Kotwal¹

¹Division of Veterinary Public Health and Hygiene FVSc & AH, R.S.Pura,

²Division of Veterinary Epidemiology and Preventive Medicine FVSc & A.H, R.S.Pura, SKUAST, Jammu - 181 102, India.

³Department of Veterinary Pathology College of Veterinary and Animal Sciences, CSK HP Agricultural University, Palampur, India.

(Received: 06 October 2011; accepted: 23 December 2011)

The present study was conducted to assess the faecal pollution of different drinking water sources in and around Jammu. A total of 50 water samples from tubewells (n=20), filling stations (n=15) and drinking water sources of livestock/poultry (n=15) were analysed. Tube wells, filling stations and livestock/poultry supply recorded positivity percentage of 60, 46.66 and 86.66 for Coliform, 55, 53.33 and 66.66 for faecal *Streptococcus* and 45, 40 and 53.33 for *Clostridium perfringens* respectively. *E. coli* was demonstrated in 35, 33.33 and 53.33 tube wells, filling stations and livestock/poultry supply and based on WHO, BIS and/or ICMR standards for coliform count 60, 46.66 and 86.66 per cent samples, respectively, were non potable.

Key words: Drinking water, Tube wells, Filling stations, Livestock, Poultry and Jammu.

The human body comprises approximately 70% of water, making it most necessary for life and good health. Water is undoubtedly the most precious natural resource that exists on our planet, without which life on earth would have been not possible. Good quality water is odourless, colourless, tasteless and free from faecal pollution.

The demand for good quality water for drinking and other purposes is no doubt exceeding the supply. It has been estimated that a minimum of 7.5 litres of water per person per day is required in home for drinking and preparing food. About 50 litres per person per day is needed to ensure all personal hygiene, food hygiene, domestic cleaning and laundry needs. This domestic water consumption is dwarfed by the demands of agriculture and ecosystem.

The presence of coliforms, faecal *Streptococci* and *Clostridium perfringens* in water is indicative of continuous pollution, rendering the water unsuitable for consumption (Hutchinson & Ridgway, 1977; Pathak & Gopal, 2001). Water for drinking purposes is required to meet certain standards (Fonseca *et al.*, 2000). The bacteriological examination of water therefore, seems to be the only powerful and foremost tool to foreclose the presence of microorganisms that might constitute a health hazard (Bonde, 1977).

* To whom all correspondence should be addressed.
E-mail: asifent2008@gmail.com

The physico-chemical and microbiological analyses of surface and groundwater becomes a necessity to arrive at a meaningful impact assessment of domestic and industrial activities on our water bodies (Amund and Odubella, 1991). The determination of the Most Probable Number and Standard Plate Count of these organisms by multiple tube fermentation technique and pour/spread plate method respectively, in water as such serves as yardstick for water hygiene surveillance (Edberg *et al.*, 2000; Ashbolt *et al.*, 2001). Provision of supplying drinking water free from micro organisms is the first task for introduction of environmental sanitation and hence analysis of water for its bacteriological quality is of paramount importance (Guerrant *et al.*, 1999). *E. coli* is the faecal indicator of choice used in WHO Guidelines for Drinking-water Quality because it gives indication of faecal contamination. Coliform bacteria may not be directly related to the contamination of water; however, their existence in drinking water suggests the potential presence of pathogenic enteric microorganisms such as *Salmonella* spp., *Shigella* spp. and *Vibrio cholerae* etc. Coliform bacteria are thus considered the best indicator of faecal pollution and the presence of pathogens.

The increasing cases of waterborne diseases in recent times necessitated the investigation of drinking water sources of Jammu region. The magnitude of problem associated with unsafe drinking water has not been systematically assessed in this part of state; although newspapers occasionally bring in focus some reported water borne illnesses. The assessment of hygienic status of drinking water goes a long way in reducing the burden of water borne diseases. Keeping in view the widespread pollution of water, the assessment of hygienic quality in different drinking water sources of Jammu was studied.

MATERIALS AND METHODS

The samples collection was carried out following the standard procedure as outlined by World Health Organisation (2008). A total of 50 water samples, 300 ml in quantity were collected in sterilized neutral glass bottles provided with ground glass stoppers and the neck protected by aluminium foil. The samples were collected randomly from tube wells of different localities (rural and urban),

filling stations (rural and urban), drinking water supplies of livestock and poultry. Twenty samples were collected from tube wells, (10 each from rural and urban localities). Fifteen samples were collected from the filling stations (8 from rural and 7 from urban localities) and the 15 samples were collected from different drinking water sources of livestock and poultry. The samples were collected in sterile containers (Hi Media, Ltd Mumbai, India) and transferred to laboratory over ice within 2-3 hours of collection or stored at refrigeration temperature for processing in any case within 6-8 hr of collection.

The samples were processed for estimation of most probable number of index bacteria using standard procedure for Multiple Tube Fermentation Technique (W.H.O. 2008). Fifteen tube dilution procedure was followed for enumeration of most probable number of coliforms, faecal *Streptococci* and *Clostridium perfringens* using double and single strength bromo-cresol purple MacConkey's bile broth, Hanny & Norton's sodium azide broth and Litmus milk respectively (Hi Media Ltd., Mumbai, India). The serially diluted samples were processed for determination of SPC employing surface spread plate technique on plate count agar (Daine *et al.*, 1995). Isolation and identification of the organisms was carried out as per the method described by Cowan and Steel (1993).

RESULTS

MPN of indicator organisms in tube wells and filling stations

A total of twenty samples from tubewells (10 each from rural and urban areas) were analysed for total coliforms, faecal coliforms, faecal *Streptococcus* and *Clostridium perfringens* in both rural and urban tube wells (Table 1). The counts of coliforms, faecal coliforms, *Clostridium perfringens* did not differ significantly while faecal *Streptococcus counts* differed significantly for rural and urban tube wells. Similarly a total of 15 filling station samples (8 and 7 samples each from the rural and urban filling stations) were analysed. The level of contamination with respect to total coliforms, faecal coliforms, faecal *Streptococcus* was found similar in both rural and urban filling stations as they did not differ significantly

Table 1. MPN (Mean \pm SE) of Indicator Organisms present in Tube wells and Fillings Station (Rural & urban)

Source		No . of samples	Indicator organisms			
			Total organisms	Feacal coliforms	Faecal Streptococcus	Cl. perfringens
Tube wells	Rural	10	25.20 \pm 9.91 (0-70) *	8.50 \pm 4.34 (0-31) *	11.4 \pm 4.7 (0-45) *	9.5 \pm 2.41 (0-22) *
	Urban	10	33.3 \pm 11.51 (0-94) *	10.1 \pm 4.60 (0-34) *	45.6 \pm 14.29 (0-138) *	11.40 \pm 2.20 (0-22) *
Filling stations	Rural	8	30.62 \pm 14.40 (0-94)*	7.62 \pm 3.76 (0-23)*	34.5 \pm 16.32 (0-109)*	5.37 \pm 1.92 (0-15)*
	Urban	7	36.28 \pm 12.96 (0-79)*	7.71 \pm 3.24 (0-21)*	33.42 \pm 12.57 (0-79)*	11.42 \pm 1.13 (6-14)*
Tube wells		t-value (P-value)	.533 (.601)	.253 (.803)	2.270 (.036)	.581 (.569)
Filling stations		t-value (P-value)	.288 (.778)	.018 (.986)	.051 (.960)	2.607 (.022)

*indicate the range, Numbers in the same column do not differ significantly (P>0.05)

Table 2. MPN (Mean \pm SE) of indicator organisms present in water from livestock/poultry supply.

Source of water samples	No. of samples (n=15)	MPN (Mean \pm SE) per 100ml water			
		Total Coliforms	Faecal Coliforms	Faecal <i>Streptococcus</i>	<i>Clostridium perfringens</i>
Livestock & poultry water supply.	15 (0-221)	91.40 \pm 15.73 (0-94)	23.13 \pm 7.68 (0-175)	67.33 \pm 18.53 (0-221)	57.26 \pm 22.34

Table 3. Standard Plate Count of tubewells, filling stations, livestock and poultry water supply

Source	No of samples	Colony Forming Units (CFU) per ml	t value (P value)
Tube wells	Rural	10 1.16x10 ⁵ (1.14x10 ⁴ -2.12x10 ⁵) *	.480 (.637)
	Urban	10 1.38x10 ⁵ (1.23x10 ⁴ -2.52x10 ⁵) *	
Filling stations	Rural	8 1.003x10 ⁵ (1.10x10 ⁴ -2.11x10 ⁵) *	.549 (.601)
	Urban	7 1.24x10 ⁵ (1.15x10 ⁴ -2.22x10 ⁵) *	
Drinking water source of livestock and poultry	15	1.56x10 ⁵ (2.42x10 ⁴ -2.31x10 ⁵) *	-

*indicates the range

however, *Clostridium perfringens* counts showed significant difference with mean value being higher in urban filling station (Table 1).

MPN of indicator organisms in livestock/poultry supply

A total of 15 livestock/poultry supply water were analysed. The counts for total coliforms, faecal coliforms, faecal *Streptococcus* and

Clostridium perfringens of livestock/poultry supply are depicted in Table 2.

Standard Plate Count

In tube wells and filling stations, comparable SPC was observed which was lower to that of livestock and poultry water supply (Table 3).

Table 4. Positivity of samples for Indicator Organisms in different drinking water sources

Source	No. of samples	Indicator organisms			
		Coliforms n=50	<i>E.coli</i>	Faecal <i>Streptococcus</i>	<i>Clostridium perfringens</i>
Tube wells	20	12 (60)	7 (35)	11 (55)	9 (45)
Filling stations	15	7 (46.66)	5 (33.33)	8 (53.33)	6 (40)
Livestock and poultry drinking water supply	15	13 (86.66)	8 (53.33)	10 (66.66)	8 (53.33)

DISCUSSION

The presence of number of indicator bacteria of coliforms, faecal coliforms, faecal *Streptococcus* and *Clostridium perfringens* of tubewell water was comparatively higher than permissible values. Forty per cent of the household water was potable based on WHO/BIS/ICMR standards for coliform. The present findings are similar to (Aydin, 2007; Goel *et al.* 2007). Alzahrani and Gherbawy (2011) reported 86.7 per cent of groundwater sources of Saudi Arabia contaminated with *E. coli*.

The MPN of the faecal *Streptococcus* in present study was higher than coliforms, faecal coliforms or *Clostridium perfringens* in groundwater and our findings are in agreement with Geen *et al.* (2011). The faecal *Streptococcus* were detected more often than thermotolerant coliforms (*E. coli*) while it may be due to higher numbers in faecal material than other bacteria besides being more resilient in non-enteric environments. The present findings of *Clostridium perfringens* counts are in accordance with the

finding of Willayat *et al.* (2005) who recorded the lowest occurrence of *Clostridium perfringens* in well water samples in Srinagar, Jammu & Kashmir. The coliforms, faecal coliforms, *Clostridium perfringens* counts did not differ significantly in water samples from rural and urban tubewells. However, faecal *Streptococcus* counts differed significantly being higher in urban areas. It may be due to point source of contamination near the tube well.

The Standard Plate Count (SPC) of tube-wells in present study in rural and in urban areas concurred with the findings of Adeyemo *et al.* (2002). The lower SPC counts from tube well water compared to the Tawi river water may be attributed to the nutrient deficient under-ground aquatic environments. The tube wells do not normally receive allochthonous inputs thus are deficient in nitrogen and phosphorus and this has been reported to account for the decreased recovery of bacteria from these sources (Hill and Rai, 1982). It may also be due to filtration effect of different layers of earth.

The positivity percentage of coliforms,

faecal coliforms, faecal *Streptococcus* and *Clostridium perfringens* in filling stations were 46.66, 33.33, 53.33 and 40 respectively. Similar findings were also reported by Jayadev and Thanga (2010). The level of contamination with respect to total coliforms, faecal coliforms, faecal *Streptococcus* was found similar in both rural and urban filling stations as they did not differ significantly ($P < 0.05$). However, *Clostridium perfringens* counts showed significant difference with mean value being higher in urban filling stations than rural filling stations. The presence of *Clostridium perfringens* in water indicates long standing pollution of water and the co-presence of *E.coli* and faecal *Streptococcus* along with it shows continuous pollution in water supply.

Filling stations from rural areas showed lower SPC/ml count than urban filling stations. Our findings corroborate with the findings by Jayadev and Thanga (2010). The recorded estimate of SPC for filling station was lowest among the untreated water. The ground water sources are often used without any treatment, except physicochemical ones to reduce hardness or eliminate off-flavours and odours. Active inspection, surveillance and preventive maintenance will all be required for sustainable drinking water management and safety assurance.

The percentage of coliforms, faecal coliforms, faecal *Streptococcus* and *Clostridium perfringens* in drinking water sources of livestock and poultry were found to be 86.66, 53.33, 66.66 and 53.33 respectively. Only 13.34 per cent of samples were potable for the livestock and poultry based on WHO/BIS/ICMR standards for coliform. Pathogens from animal faeces may enter waterways by direct deposition or as a result of overland runoff containing faecal material deposited in the watershed.

The MPN index of coliforms was highest, followed by faecal *Streptococcus*, *Cl.perfringens* and faecal coliforms. *E.coli* was present in 53.33 per cent of the samples from livestock and poultry water supply. The results corroborate with the findings of Jafari *et al.* (2006). The findings of SPC in drinking water of livestock/poultry are also in agreement with the earlier report of Nasrin *et al.* (2007). Water derived from surface water showed increases in most of the investigated bacteriological parameters, followed by traditional sources

(tubewells and filling stations) as compared to post filtration and household water supply. This may be attributed to the fact that well and surface water are at risk of contamination as indicated by the higher levels of most bacteriological parameters. Moreover, well water is exposed to point sources of pollution such as septic wells and domestic and farming effluents, as well as to soil with high humus content. The lower bacteriological characteristics in samples from post filtration and household water supply indicate that it is satisfactory for human drinking purposes. Contamination of household water may occur during transportation from the treatment plant or in the house reservoirs of the consumers. Improving and expanding the existing water treatment and sanitation systems is more likely to provide safe and sustainable sources of water on long term basis. Strict hygienic measures should be applied to improve water quality and to avoid deleterious effects on public health, by using periodical monitoring programmes to detect faecal pollution.

REFERENCES

1. Alzahrani, A. M. and Gherbawy, Y. A. Antibiotic resistance in *Escherichia coli* strains isolated from water springs in Al-Ahsa Region. *African Journal of Microbiology Research*, 2011. **5**: 123-130.
2. Adewoye, S. O., Ogundiran, M. A. and Adebayo, E. A. Physico Chemical and Bacteriological quality of some vended sachet water samples in Ilorin, Nigeria. *Journal of Research in Biology*, 2011. **2**: 122-128.
3. Amund, O. O. and Odubella, M. T. Coliform bacteria and faecal steroids as indicators of water quality. *Proceeding of First National Conference on Water Quality Monitoring and Status in Nigeria, Kaduna*. 1991. pp 216-224
4. Ashbolt, N. J., Grabow, O. K and Snozzi, M. Indicators of microbial water quality. *In Water Quality: Guidelines, Standards and Health*; 2001 Fewtrell, L., Bartram, J., Eds.; World Health Organization (WHO), IWA Publishing: London, UK; pp 289-316.
5. Aydin, A. The microbiological and physico-chemical quality of groundwater in West Thrace, Turkey. *Polish Journal of Environmental Studies*, 2007. **16**: 377-383.
6. Bonde, G. J. Bacterial indication of water pollution. *Advanced Aquatic Microbiology*, 1977. **1**: 273-364.

7. Cowan, S. T. and Steel, S. *Manual for identification of medical bacteria*.1993. Edited by:Barrow G I, Feltham R KA. Cambridge University Press, London.
8. Daine, R., William, H. and Melody, G. *Practical Food Microbiology*,1995. 2nd Ed. Public Health Laboratory Services, London.
9. Edberg, S. C., Rice, E. W., Karlin, R. J. and Allen, M. J. *Escherichia coli: the best biological drinking water indicator for public health protection. Journal of Applied Microbiology*,2000. **29**: 106-116.
10. Fonseca, L. F. L. Concentration of hardness, alkalinity and nitrate in Water used for cleaning milk equipment in Brazilian dairy farms. *Proceedings of 10th Science Congress*. pp. 100 – 103, Maastricht, Netherlands. Geen, A. V., Ahmed, K. M., Akita, Y. and Alam, M. J. 2011.Fecal Contamination of Shallow Tubewells in Bangladesh Inversely Related to Arsenic. *Environmental Science and Technology*,2000. **45**: 1199–1205.
11. Goan, H. C., Burcham, T. N., Denton, P. H. and Draughon, F. A. Quality of well water on Tennessee poultry farms. *Poultry Science*,1992. **71**: 103.
12. Goel, K. N., Bhatnagar, T., Sharma, M. K., Verma, A., Agarwal, N., Chander, J.,Gupta, V. and Swami, H. M. Surveillance of Quality of Drinking Water. *Bahrain Medical Bulletin*,2007. **29**: 18-21.
13. Guerrant, D., Moore, S., Lima, A., Patrick, P., Schorling, J. and Guerrant, R. Association of early childhood diarrhoea and cryptosporidiosis with impaired physical fitness and cognitive function 4 to 7 years later in a poor urban community in northeast Brazil. *American journal of Tropical Medicine and Hygiene*,1999. **61**(5): 707-713.
14. Hill, G. and Rai, H. A preliminary characterization of the tropical lakes of the central Amazon by comparison with polar and temperate systems. *Archeological Hydrobiology*,1982. **96**: 97-111.
15. Hutchinson, M. and Ridgway, J. W. *Microbiological aspects of drinking water supplies*.1977. In Aquatic Microbiology, F. A. Skinner and J. M Shewan (eds.), Academic Press. London, pp.179.
16. Jafari, R. A., Fazlara A. and Govahi, M., An Investigation into *Salmonella* and Fecal Coliform Contamination of Drinking Water in Broiler Farms in Iran. *International Journal of Poultry Science*,2006. **5**: 491-493.
17. Jayadev, A. and Thanga, V. S. G. Seasonal changes in coliform contamination of potable ground water sources in Thiruvananthapuram , Kerala, India. *Asian Journal of Environmental Science*,2010. **4**(2):181-183.
18. Nasrin, M. S., Islam, M. J., Nazir, K. H. M. N. H., Choudhury, K. A. and Rahman, M. T. Identification of bacteria and determination of their load in adult layer and its environment. *Journal of the Bangladesh Society for Agricultural Science and Technology*, 2007. **4**: 69-72.
19. Pathak, S. P. and Gopal, K. Rapid detection of *E.coli* as an indicator of faecal pollution of water. *Indian Journal of Microbiology*,2001. **41**: 139.
20. W. H. O. *Guidelines for Drinking-water Quality* , 2008. Third Edition Incorporating the First and Second Addenda Volume 1 Recommendations. W.H.O .Geneva.
21. Willayat, M. M., Hussain, S. A and Nabi, A. Bacteriological analysis of stream, tube well and community supply water in and around Srinagar. *Indian journal of Comparative Microbiology, Immunology and Infectious Diseases*,2005. **26**: 66-67.