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Research Article

Prevalence of Thermophilic *Campylobacter* Isolated from Water Used in Slaughter House of Kathmandu and Ruphendehi District, Nepal

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Abstract

Campylobacter is a fastidious organism that is capable of surviving in wide range of environments and potentially can cause serious infection in human and animal which can commonly available in the different sources of water used for daily purpose. This research was conducted to figure out the prevalence of the thermophilic *Campylobacter* in the water used in various slaughter houses of Kathmandu and Ruphendehi district of Nepal. So a cross sectional study was conducted and 200 water samples (100 for each district) was collected aseptically and submitted to Bacteriological Unit for the confirmation. Isolation and identification of *Campylobacter* was being done as described by OIE Terrestrial Manual, 2004, Chapter 2.8.10. Laboratory finding was done to confirm the positive cases. The study revealed the prevalence status of *Campylobacter* in water used in the slaughter house of Ruphendehi district is 12% and Kathmandu Valley is 0.00%. Thus, Ruphendehi has comparatively more prevalence of *Campylobacter* than Kathmandu. The water samples tested were all from actual or potential water sources for the use in slaughter house, so there are clear implications for the transmission of *Campylobacter* spp. to human and animal suggesting further expanded research is required in this area.

Keywords: *Campylobacter*; prevalence rate; cross sectional study; slaughter house

Introduction

Livestock sector is an important component of Nepalese economy in terms of income, employment and equality. Livestock sector contribute one-third of agricultural GDP and 4% of the total export of the nation (Osti *et al.*, 2015; Osti *et al.*, 2016; MOAC, 2016). Meat production in Nepal is one of the major and most profitable businesses of agriculture that provides nutrition for human consumption with in shortest possible time. No modern slaughter house except one at Hetuada constructed about 20 years ago. Animal slaughtering occurs at street sides, river sides, open pasturelands and courtyards (Joshi, 2008). With a shift in consumption pattern from conventionally well cooked meat to ready to eat meat item, increased the risk of food zoonosis, particularly *Campylobacter*iosis and

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Salmonellosis. Fig. 1 shows the contribution of meat production in Nepal.

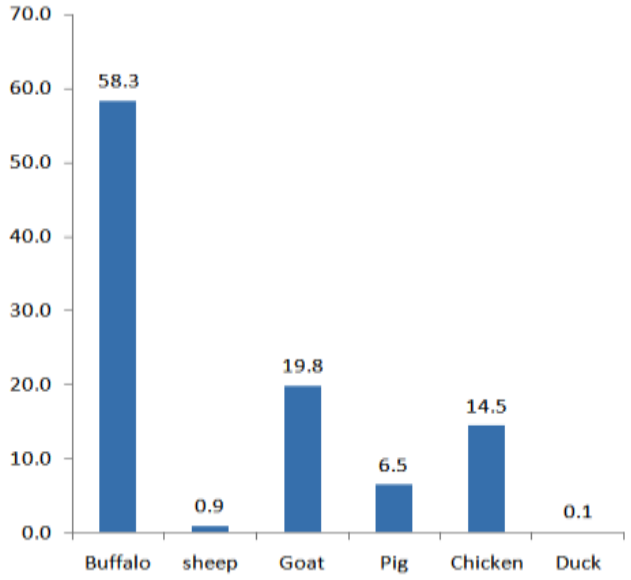

Fig. 1: Contribution of Meat Production in Nepal (Source: MoAC, Nepal, 2017)

In last 25 year, *Campylobacter jejuni* and *Campylobacter coli* account for the majority of human infections (Friedman *et al.*, 2000) and are commonly referred to as "thermophilic" *Campylobacter*'s, being able to grow at 37 °C to 42 °C with an optimum growing temperature of 42 °C but incapable of growth below 30 °C. *Campylobacter* is a commensal organism routinely found in cattle, sheep, swine, goats, dogs, cats, rodents, and fowls.

Campylobacter is a leading cause of food borne diarrhoeal illness worldwide primarily associated with *Campylobacter* jejuni and *Campylobacter* coli (Zhao *et al.*, 2015; Skarp *et al.*, 2016). Consumption of *Campylobacter*-contaminated poultry meat is the main cause of *Campylobacter*iosis infection, which makes poultry-slaughtering lines a key link in *Campylobacter* risk assessment (Huang *et al.*, 2018).

Campylobacter species are Gram-negative spiral, rod-shaped, or curved bacteria with a single polar flagellum, bipolar flagella, or no flagellum, depending on the species (Man, 2011) having dimensions of 0.2-0.8 µm width and 0.5-5 µm length. These bacteria are microaerophilic, but some can grow aerobically and anaerobically. They neither ferment nor oxidize carbohydrates (Kaakoush, 2015). Some species, particularly *Campylobacter jejuni, C. coli, C. lari, and C. upsaliensis* are commonly referred to as thermophilic *Campylobacter*, being able to grow at 37-42ºC with an optimum growing temperature of 42.0ºC but incapable of growing below 30.0ºC.

It is obligatory for the slaughter house to follow the provisions of 'Slaughter House and Meat Inspection Act-2055', to assure their consumers that the products are safe for human consumption. The problem of *Campylobacter* contamination in water must be addressed to date; no practical or effective control measures have been available (Newell and Wagenaar, 2000). Butchers and poultry farm workers are at high risk for human *Campylobacter*iosis. Improper handling and un-boiled water leads to incidence of human *Campylobacter*iosis that is the final sequel of fecal contamination of carcass. Understanding the status of *Campylobacter* contamination in the water is very important. Various studies outside Nepal have demonstrated evidence and prevalence of the disease due to contaminated water and meat. It has been reported that recovery of thermophilic *Campylobacter* from chicken is approximately six times higher than from pork or beef or mutton (Shane, 2000).

In Nepal, there has not been any documented work on the occurrence of *Campylobacter* in water. The fact that contaminated water being the major contributor of human infection cannot be neglected in the countries like Nepal. This study thus includes the retail meat shop water which may prove the cause of transmission to human and other animals. Similarly, prevalence of food born Zoonosis by *Campylobacter* and antibiotic sensitivity is most important concern worldwide.

Materials and Method

Study Sites

The study was conducted at Veterinary Microbiology Laboratory of the IAAS, Rampur. The samples were collected from different locations of Kathmandu Valley and Ruphandehi district.

Sampling and Sample Collection

With the confidence interval of 95.0% and maximum allowable error of five percent, the total sample size was determined using software Win Episcope Version 2.0 (EPIDCON). The expected prevalence of *Campylobacter*iosis is 20.0% used for sample size determination using the following formula for Win Episcope

$$SD = \sqrt{p*(1-q)}$$
$$=\sqrt{0.20*(1-0.20)}$$
$$=0.3577$$

Where p is the expected prevalence of 20.0% and sample size n is calculated using the above standard deviation (SD).

$$n = [t*SD/L]^2$$
$$= [1.96*0.3577/.05]^2$$
$$= 196.62 \approx 197$$

Where t is Student's t-value of 1.96 with the desired level of 95.0% confidence and L is the allowable absolute error of five percent keeping 3 samples size as risk in preventing

sample loss during analysis, the total sample size was estimated at 200 samples. The collected sample water was transported to the Veterinary Microbiology Laboratory of Rampur Campus, IAAS, maintaining cold chain with the help of icebox and was stored at refrigerator (2-8ºC) if necessary. The individual slaughter house was randomly selected. Individual water sample from individual slaughter house was collected. Sampling bottle 100 ml capacity, transparent sterilized by 70.0% alcohol was used to collect the sample.

Storage and Transport of Stocks Cultures

The identified *Campylobacter* was inoculated in BHI broth (M210, HiMedia lab, Mumbai, India) tubes with loosen screw caps and was incubated for 48 hrs at 42ºC in anaerobic jar with lighting candle. It was then added with equivalent amount of 40.0% glycerol to make final 20.0% in screw cap stock vial at -70ºC for stocking.

Enrichment and Primary Culture

Aseptically collected samples water was kept in icebox/refrigerator and thaw prior to culture. One volume of the sample water was added to nine volume of Bolton broth (CM0983, Oxoid ltd., Basingstoke, Hampshire, England) for enrichment. The inoculated broths was incubated in microaerophilic atmosphere obtained by burning candle in candle jar system (BD1777SE, Don Whitely scientific ltd, England) for microaerophilic culture at 42ºC for 48 hrs. Following incubation, one loopful of broth culture was streaked on plate modified CCDA (mCCDA) using the four quadrant streak method for isolation. Inoculated plates were incubated at 42ºC in a microaerophilic atmosphere for 48 hrs using candle jar (BD1777SE, Don whitely scientific ltd, England). *Campylobacter* suspected colonies were subculture one or more times, until monoculture was obtained. Colonies which appeared gray, flat and moistened with the tendency to spread and have metal sheen will be identified as *Campylobacter*. More precisely these typical colonies were subculture onto tryptose blood agar (TBA) plates under similar conditions as above. Pale yellow colonies with mucoid appearance stained by modified Gram's staining for presumption of *Campylobacter*. The *Campylobacter* are Gram's negative and curved bacilli. The presumptive *Campylobacter* colony resulting from respective plates was identified based on Gram's staining reaction, cellular morphology, cultural characteristics and biochemical reactions.

Identification of Campylobacter spp.

On modified charcoal cefoperazone deoxycholate agar (mCCDA)

Campylobacter colonies on mCCDA appears as smooth, shiny and convex with a defined edge or flat, transparent or translucent and spreading with irregular edge, colorless to grayish or light cream, usually 1-2 mm in diameter.

Gram's staining

The Gram's stain involves a different staining utilizing several stains. The primary stain is crystal violet, second a solution of iodine as a mordant which react with the primary stain and cell components to enhance the reaction of the primary stain. This causes purple insoluble complexes to form with the cells RNA. Then the procedure involves rinsing with alcohol or acetone and finally with some counters stain like safranin. Gram positive lose the crystal violet color when treated with the decolorizer and are counter stained by safranin and hence appear red or pinkish red in color. The *Campylobacter spp* appears Gram negative and small curved rod on microscopic observation.

Potassium hydroxide test

A loopful of the culture was mixed with an equal amount of three percent potassium hydroxide on a clean microscopic slide. After through mixing the loop was lifted at intervals to see whether a gel was formed. The *Campylobacter spp*. are positive to this test and show a viscous gel within 60 secs. It indicated that the organism was Gram negative bacteria.

Catalase test

A loopful of bacterial growth was taken from the tip of the colonies avoiding the blood agar medium. The bacterial cells were placed on a clean microscopic slide and a drop of three percent hydrogen peroxide was added. An effervescence of oxygen gas, within few sec indicates a positive reaction. *Campylobacter spp*. showed positive to this test.

Oxidase test

Commercially available oxidase discs were moistened with sterilized distilled water. Using a sterile glass rod, colony to be tested will be smeared on the oxidase disc. If the bacteria oxidaze the reagent (remove electrons) the smeared area truns dark purple within 30 secs, indicating a positive test. The *Campylobacter spp* was positive to this test and show color changes.

Triple sugar iron (TSI) agar

The TSI agar was prepared as slant and butt in tubes. The TSI agar contains fermentable sugars (0.1% glucose, 0.1% lactose and 0.1% sucrose), ferrous sulphate to indicate hydrogen sulphide production and pH indicator (phenol red). Organism that ferments glucose only produces alkaline (red) slant and acid (yellow) butt. Organism that ferments glucose as well as lactose or sucrose produces yellow (acid) slant and yellow (acid) butt. Bubbles or cracks was indicating production of gas from glucose. The *Campylobacter spp*. gaves a red slant, red butt and do not produce hydrogen sulphide when inoculated into TSI.

Statistical Analysis

Data entry, management and analysis was done using program Microsoft Office Excel 2003. Total number of

positive samples from which *Campylobacter* was identified and isolated was compared by simple statistical techniques.

Results and Discussion

Contamination of water sample for Bacteria

A total of 200 water samples were collected and tested to find out the prevalence of the *Campylobacter*. Out of 200 samples (100 from each site) collected from different slaughter housed in Kathmandu Valley and Ruphendehi District, tested for the bacteriological examination, only 92 samples of Kathmandu Valley and 66 samples of Ruphendehi district showed positive growth on Nutrient Agar Media, showing contamination of different bacteria (Table 1).

Sources of Water

Among various sources of water the most common was tap water (63+86=149; 79.5%), supplied by Municipality, however other sources of water were tube well, well, river water and water jar (Table 2).

Confirmation of Campylobacter

All the Bolton Broth Enriched, water sample were inoculated on the mCCDA for the growth of the *Campylobacter*, however only 14 samples of Ruphendehi showed growth on mCCDA, out of which only 12 samples were confirmed for the *Campylobacter*. None of the sample from the Kathmandu showed growth on the mCCDA, show no sample is confirmed for *Campylobacter* (Table 3). The study showed 12% prevalence of *Campylobacter* in Ruphendehi district. Out of 12 sample of +ve cases, 10 samples (83.33%) were from the tap water (Municipality supply), 1 sample (8.33%) from tube well and 1 sample (8.33%) from water jar (Fig. 2).

Table 1: Bacterial Growth in Nutrient Agar.

S.N.	Sample	Total	Bacterial Growth (Nut. Agar)	% of Growth	No Growth	% of no Growth
1.	Kathmandu Valley	100	92	92.0%	08	8.0%
2.	Ruphendehi	100	66	66.0%	34	34.0%
3.	Total	200	164	82.0%	36	18.0%

Table 2: Sources of water in two different sites

S.N.	Sources	Kathmandu Valley	%	Ruphendehi	%
1.	Tap Water	63	63%	86	86%
2.	Tube well	13	13%	7	7%
3.	Well	9	9%	-	-
4.	River water	-	-	3	3%
5.	Water Jar	15	15%	4	4%
	Total	100		100	

Table 3: Total number of *Campylobacter* Growth

S.N.	District	Total Sample	Positive for *Campylobacter*	Prevalence of *Campylobacter*
1.	Kathmandu Valley	100	0	0.00%
2.	Ruphendehi	100	12	12.00%

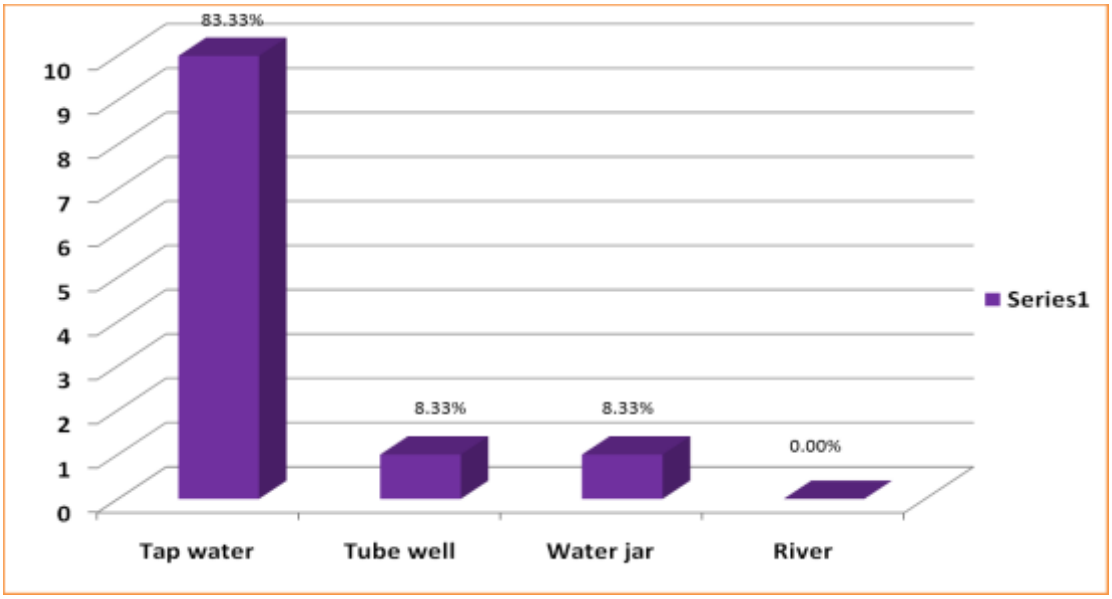

Fig 2: Various sources of *Campylobacter* contaminated water

This is the first report of isolation of *Campylobacter spp*. from water sample in Ruphendehi district of Nepal. The present study demonstrated that, prevalence of *Campylobacter* spp. was 12.0%. The prevalence is lower than the study done by S. M. Diergaardt *et al.*, 2002, (13.6%) in the water sources in South Africa. Similarly *Campylobacter* spp. in waters of three main Western Pomerania water bodies done by Department of microbiology, Agriculture University, Szczecin, Poland showed presence of *Campylobacter spp*. at the level detectable by the method applied, was confirmed in 19.7% in the Odra River (greater than present study), 5.6% of the Szczecin lagoon (lower than present study) and 0% (lower than present study) of the Pomeranian Bay surfaces water samples. Environmental factors were found to be associated with the presence of *Campylobacter* spp. in water. The probability of isolating *Campylobacter* spp. in water samples from tap water was greater than in those from other sources. The reason for this presence of *Campylobacter* spp. in water is unclear. Ghimire *et al.* (2014) found the prevalence of various types *Campylobacter* 38.85% in the Pork of their study area, which is significantly greater than the present study. These differences may be due to slaughtering practices, antibiotic usage, or intrinsic carriage rates. Some of the differences in prevalence rates may also reflect differences in methods used to culture the *Campylobacter*.

Conclusion

The *Campylobacter spp*. is prevalent in the water samples. The present study showed that *Campylobacter spp*. commonly contaminates water of retail meat shop. Relatively high prevalence and contamination levels present a potential risk to public health. It is likely that this risk is controlled only by proper hygiene and sanitation during meat processing especially to minimize fecal contamination. *Campylobacter* is a frequent cause of diarrhoea/ dysentery in children in our set up. It accounts for as much as 20% of the etiology of bacterial diarrhea in Nepal. *Campylobacter* spp. was commonly isolated from water in this study. The water samples tested were all from actual or potential water sources for the use in slaughter house, so there are clear implications for the transmission of *Campylobacter* spp. to human and animal. Direct human exposure is also possible within the study area, as it is widely used for recreational and consumption purposes. Among various sources of water, Tap water (Municipality supply) is the most common (83.33%) for *Campylobacter* prevalence so concern government authority need to take quick attempt to prevent the *Campylobacter*osis in human. Water for consumption by human needs roll boil, for the prevention of *Campylobacter*.

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