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# A survey of *campylobacter* species in broiler chicken sold in Kampala markets, Uganda

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## Abstract

**The survey was conducted to establish the presence and extent of contamination of *Campylobacter* species in broiler chicken sold in Kampala. One hundred (100) samples of market broiler chickens were randomly collected from supermarkets, local markets, butcheries, and a poultry-processing plant in and around Kampala. Presence of *Campylobacter* species in the samples was determined using direct plating technique onto Campylobacter selective media containing Cefoperazone. *Campylobacter* species were isolated from 89 of 100 samples (89%) of which 11% were from birds collected from supermarkets, 18% from local markets, 20% from butcheries and 40% from a processing plant.**
**There is therefore need for intervention measures to reduce these levels of contamination in order to protect the public against campylobacterosis. These would include among others, education of the public and institution of good manufacturing practices in processing plants.**

**Key Words:** Campylobacter species, broiler chicken, prevalence

## Introduction

Poultry production has continued to increase especially in the urban areas in Uganda. Estimates show that poultry (chicken) numbers increased from 21.8 million in 1995 to 23.8 million in 1996 (MPED, 1997/1998). This increase has partly been attributed to increased tourist activity in the country resulting in increased demand for eggs and poultry meat from hotels and places of entertainment (MPED, 1997/1998).

*Campylobacter* is often present on broiler carcasses and, epidemiological studies suggest there may be links between contamination of poultry by Campylobacter and human enteritis (Tauxe et al., 1985 and Harris et al., 1986).

*Campylobacter* species can cause mild to severe diarrhoea, with loose, watery stools often followed by bloody diarrhoea (Butzler, 1984). Reports show that C. jejuni, *C. coli,* and *C. lari* account for more than 99% of human isolates of which *C. jejuni* is the most common with 90% (Hunt et al., 1998). *Campylobacter* has also been implicated in travelers' diarrhoea (Pitkanen, 1982). In many countries, *C. jejuni* has been isolated from patients with diarrhoea at rates greater than for Salmonella (Tauxe et al., 1985). Hygiene interventions in the chicken processing alone are not expected to be effective because of the constant flow of bacteria into the process via many unavoidable sources of cross-contamination. These include contaminated feed and water, carcass to carcass contact, scalding, chilling, dust, flies, insects, utensils, rodents and workers' hands (Sean et al., 1999). However, this bacterial load may be reduced during other processing stages of washing and chilling or freezing. But these steps may be a source of cross-contamination to non infected carcasses (Sean *et al.*, 1999). This suggests that Campylobacter species, if present, are carried along on the processed but raw poultry and may still survive in chilled and undercooked meat.

Although the presence of *Campylobacter* in poultry causes no appreciable loss to production, many consumers, processors and farmers may not be aware of the danger of Campylobacter to human health (WHO, 1990).

*Campylobacter* is prevalent in market-age poultry, and has been frequently detected, often in large numbers, on the feathers and skin, and in digestive system of chicken prior to slaughter (Sarge, 2000). Cross-contamination occurs in the processing environment from many sources including scalding, picking, evisceration and chilling operations. As with other microorganisms, Campylobacter initially suspended in surface water film, rapidly attach or become entrapped in the poultry meat and on the skin surfaces. Once firmly attached or entrapped in skin cracks and crevices, Campylobacter are able to resist water, chlorine and other carcass biocides that are currently available for disinfection (Sarge, 2000).

Currently, there is a lot of chicken consumption in households, restaurants and entertainment centers partly due to the increased production rate from farms and processing plants (MPED, 1997/1998). In Kampala, chicken is sold live or dressed or may be in the form of ready-to-eat meat, grilled, roasted or cooked meat. Yet, if not properly cooked, there is a risk of infection with Campylobacter. However, there is a lack of information on the magnitude and hence, the risk of campylobacteriosis and the probable degree of exposure to the consumers in Uganda. The main objective of the study was to show the magnitude of Campylobacter in market broilers on Kampala market. And specifically to establish the presence and quantity of Campylobacter in broilers which are available at the distribution chain ready to market.

## Materials and methods

Using Rigney Associates Researchers' Toolbox: Sample size calculator, One hundred (100) samples were collected from supermarkets, local markets, butcheries and one processing center because market broilers are commonly sold at these outlets to the consumers. Samples from each category were collected on the same day.

Whole chicken samples were collected in sterile sampling bags and labeled accordingly. Samples were then transported under ice in an insulated container and delivered to the laboratories Data taken for all samples including time, dates of collection, state of the sample, at time of collection, was recorded. Samples were analysed within 24 hours of collection.

### Sample analysis

Samples were tested for presence of *Campylobacter* using standard methods (International Standard, ISO 10272, 1995 edition; FDA manual, 1998).

### The sample

The neck of poultry is the lowest part during processing so all fluids flow over it. From each collected broiler sample, 25g of neck skin was aseptically removed and placed in 225ml of Buffered Peptone Water in a stomacher bag and subjected to stomaching.

### The medium

Campylobacter selective Preston kit (BBL™) was used. The kit consists of Preston agar, a growth factor; and Cefoperazone (an antibiotic). The Preston agar (BBL™) was prepared according to manufacturer's instructions. Briefly, 46.5g of Preston agar were suspended in 1000ml of distilled water, agitated and boiled for one minute before sterilisation at 121°C for 15 minutes. After cooling to 45-50°C Cefoperazone and the growth factor were added (10ml each) aseptically. Media plates prepared in advance were kept under refrigeration. These would be dried at 42°C in an incubator or oven with covers removed prior to inoculation.

### Sample inoculation

For each prepared sample, 1:10 dilution was inoculated onto the Preston agar (BBL™) on the plates and spread using sterile glass rods to enable isolation of single colonies that would be counted.

### Incubation under microaerophilic conditions

Microaerophillic conditions were simulated by use of CampyPak™ gas generating envelopes (BBL™) in the anaerobic jars. The envelopes reduce the oxygen level to ~5% and produce other gases promoting Campylobacter growth. The inoculated plates were then incubated at 42°C for 24 h - 48 h before examining them for characteristic colonies of Campylobacter.

### Identification and confirmation

Colonies of suspect *Campylobacter* isolates were identified and confirmed based on characteristic colony appearance, morphology, Gram reaction, growth at 25°C, and biochemical tests (Catalase, Oxidase, Reaction on Triple Sugar Iron agar). Data generated per sample was recorded.

### Characteristic colonies

Typical colonies of *Campylobacter* appearing flat or slightly raised, spreading, with an irregular edge and grayish color were identified by physical characteristics on media.

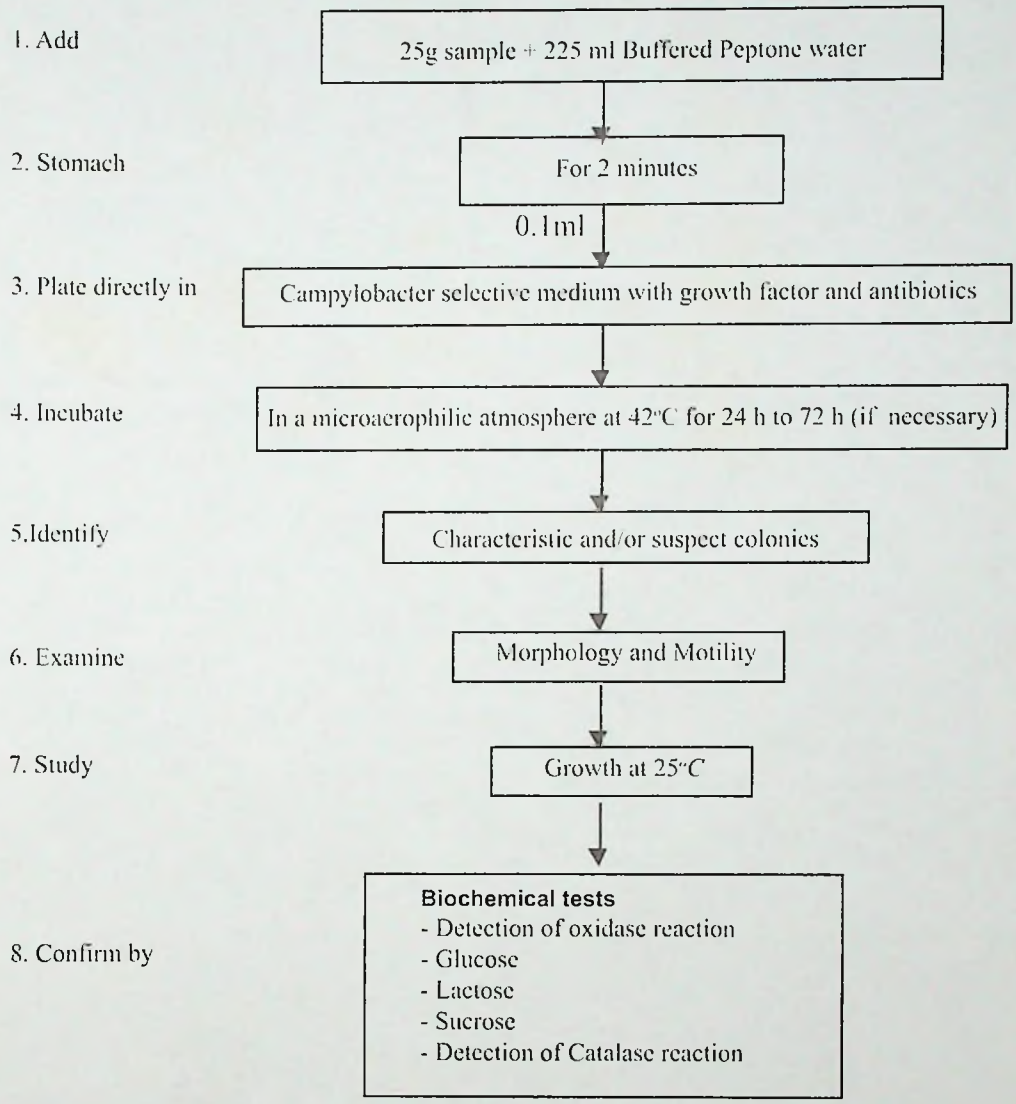
### Growth at 25°C

Characteristic colonies were inoculated in pre-prepared sterile Brucella broth and incubated at 25°C under Microaerophillic conditions.

### Biochemical tests

These included Gram reaction, Catalase, Oxidase tests and reaction on Triple Sugar Iron Agar.

## Analysis flow chart




**Reference: FDA Manual, 1988 and ISO 10272:1995(E)**

## Results

In the four supermarkets, 11 out of 20 samples collected were positive with the highest proportion of positives (4/5) in samples from supermarket D. The highest load was also found in samples collected from supermarket D as shown in Table 1.

There is a generally low distribution of Campylobacter species as seen in figure 2. Supermarket B has the lowest load (6 cfu/25g) while supermarket D has the highest load of 148 cfu/25g.

18 out of 20 samples collected from local markets of Ntinda, Kamwokya, Nakawa and Wandegeya (each five samples) were positive for Campylobacter with Kamwokya yielding the highest average load as shown in table 1 above.

The lowest average distribution in this category of markets was 42 cfu/25g from Nakawa market and the highest was Kamwokya market with 308 cfu/25g (figure 3).

All 20 samples collected from butcheries were positive. Of the four butcheries, samples from

Wandegeya yielded the highest Campylobacter species load as shown in figure 4 above. The lowest mean of 86 cfu/25g was from Ntinda butchery samples and highest (1054 cfu/25g) from Wandegeya butchery samples (Table 1).

*Table 1: Mean distribution and standard deviation of Campylobacter species in samples from the different sources*

| Source | | Mean (cfu/25g) |
|---|---|---|
| Supermarkets | A | $1.6\pm3.0 \times 10^1$ |
| | B | $6.0\pm0.9 \times 10^1$ |
| | C | $2.4\pm2.9 \times 10^1$ |
| | D | $1.5\pm1.3 \times 10^2$ |
| Local markets | Ntinda | $5.4\pm3.2 \times 10^1$ |
| | Nakawa | $4.2\pm5.2 \times 10^1$ |
| | Kamwokya | $3.1\pm5.1 \times 10^2$ |
| | Owino | $6.6\pm4.6 \times 10^1$ |
| Butcheries | Ntinda | $8.6\pm5.1 \times 10^1$ |
| | Bukoto | $1.5\pm1.3 \times 10^2$ |
| | Kamwokya | $1.3\pm0.9 \times 10^2$ |
| | Wandegeya | $1.1\pm0.6 \times 10^3$ |
| Processing plant | | $3.6+ 3.1 \times 10^2$ |

ll the 40 samples collected from the processing plant were positive for Campylobacter giving an average of 360 cfu/25g.

As shown in table 2 below, the highest mean number of Campylobacter was 360 cfu/25g found in samples from butcheries and the processing plant. The lowest mean number was 49 cfu/25g from the supermarket samples.

The results show that sample from butcheries and the processing plant yielded the highest load than from supermarkets and local markets.

## Discussion

The overall prevalence of Campylobacter among market broiler birds studied was 89%. The types of birds studied were chilled or frozen for supermarket samples; chilled for samples in processing plant; and at ambient temperatures for butchery and local market samples. However, the prevalence of Campylobacter from processing plant and butcher samples was 100% (all samples studied were positive). Conversely, samples from supermarkets showed the least prevalence of 55%.

In the Supermarkets, broiler birds are sold frozen or chilled. These state of temperatures destroy or cause stress to living organisms. However, as studies have previously indicated, Campylobacter species if present in chicken carcasses will still survive in frozen or chilled conditions. It is also possible that the prevalence would be higher in these samples since Campylobacter species have been reported to form viable-but-non-culturable (VNC) forms when subjected to stress. During power

Figure 2: Mean distribution of Campylobacter species in borier samples from supermarkets in Kampala

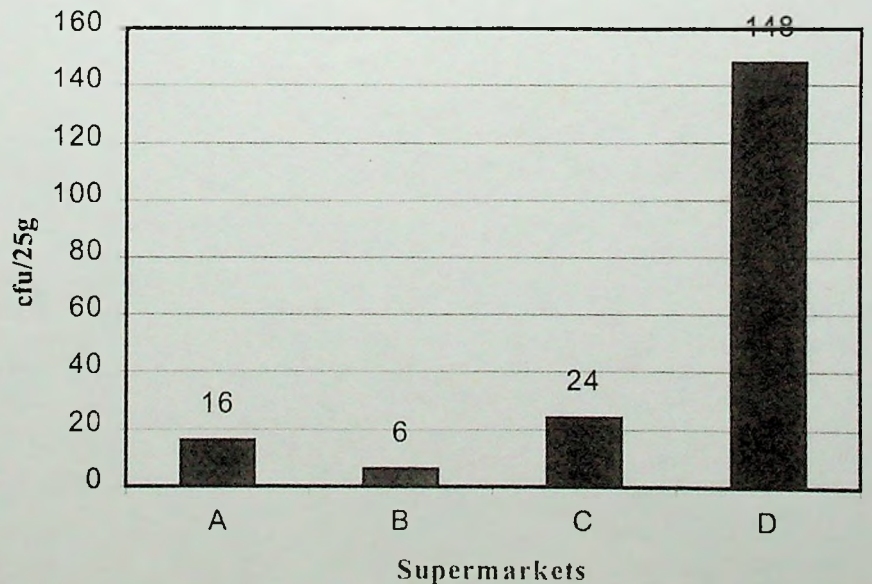


Figure 3. Mean distribution of campylobacter species in broiler samples from local markets in Kampala

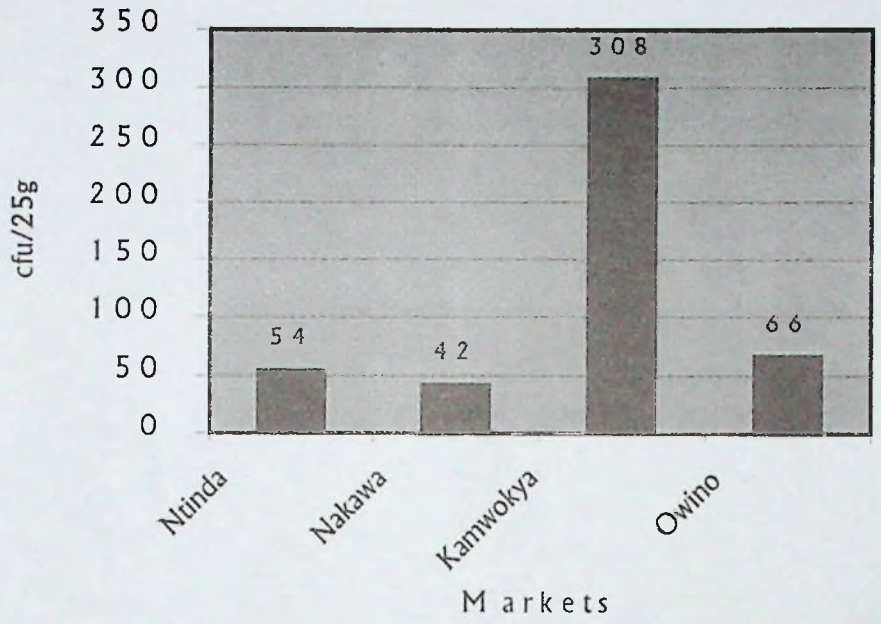



Figure 4. Mean distribution of *Campylobacter* species in broiler smaples from butcheries in Kampala

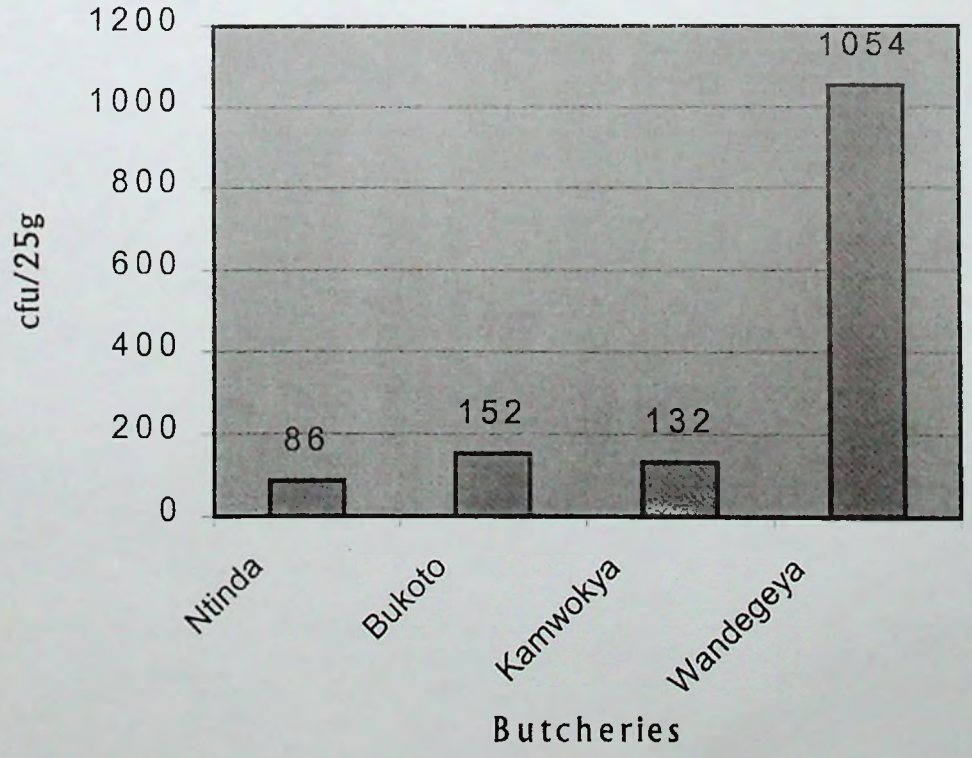


Table 2: Overall mean distribution and standard deviation of Campylobacter species in the broiler chicken in Kampala markets

| Source | Supermarkets | Local markets | Butcheries | Processing plant |
|---|---|---|---|---|
| Mean | $4.85 \times 10^1$ | $1.2 \times 10^2$ | $3.6 \times 10^2$ | $3.6 \times 10^2$ |
| Standard deviation | $8.6 \times 10^1$ | $2.6 \times 10^2$ | $4.9 \times 10^2$ | $3.1 \times 10^2$ |

Figure 5. Mean distribution of Campylobacter species in broiler samples from markets in kampala

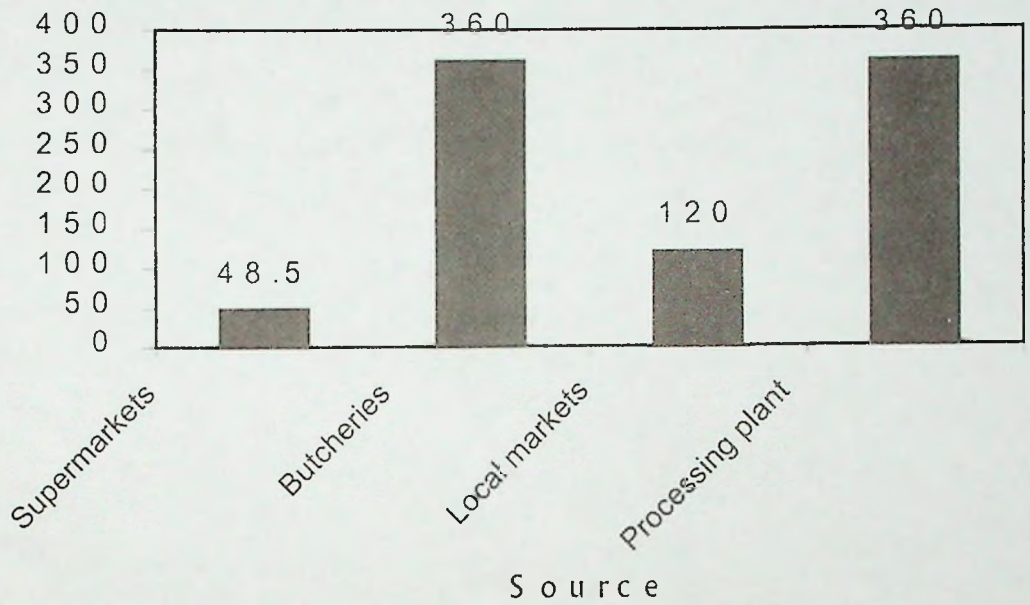



Table 3: Summary of prevalence of Campylobacter species from different market areas

| Sample source | Supermarkets | Local Markets | Butcheries | Processing Plant |
|---|---|---|---|---|
| (Sample size) | 20 | 20 | 20 | (40) |
| Number of positive samples | 11 | 18 | 20 | 40 |
| % | 55 | 90 | 100 | 100 |

load shedding sessions (say in Uganda), the chicken in freezers may begin to produce thaw liquor, which is said to have protective actions on Campylobacter species increasing their survival time.

The prevalence of Campylobacter in local market samples was 90% (18 out of 20 samples studied were positive). The broiler birds are sold live (unprocessed) and upon buying, they are slaughtered and defeathered: and some times they dress the broiler birds and display them at ambient temperatures.

In most cases the area where slaughtering and defeathering occurs is unhygienic or they use warm water in a container where they dip the carcasses to soften the feathers. Evisceration also leads to gross contamination of the carcass.

All the twenty (20) samples of broilers bought randomly from four butcheries in Kampala were positive for Campylobacter species giving a prevalence of 100% contamination.

In butcheries, chicken is sold in processed form and hanged onto hooks or display in the open stalls. Information gathered from the butcheries indicated that chickens were locally bought from markets or farmers. The method of processing was similar to that of the local markets. However, the prevalence was found to be higher probably because these chicken carcasses were left at ambient temperatures and exposed for long periods of time thus encouraging exponential growth. At a processing plant, various flocks of chicken are usually processed and packaged before distribution to the various marketing outlets.

All the hundred (100) samples studied from poultry processing plant had Campylobacter species isolated from them. The numbers (cfu/25g) were quite high probably indicating gross contamination in the processing.

The processing plant is supplied with birds from a poultry farm within the premise and contamination may arise within flocks by the infected chicken.

Also, hygiene interventions during processing alone are not expected to be effective because bacteria flow into the process via many unavoidable sources of cross-contamination. These include contaminated feed and water, carcass to carcass contact, scalding, chilling, dust, flies, insects, utensils, rodents, and worker's hands.

## Conclusion and recommendations

Uganda as a developing country, is living with Campylobacter species in broiler chicken without sufficient knowledge that it exists; or that it is a possible serious cause of gastroenteritis cases.

The occurrence of Campylobacter species in 89 out of 100 samples collected indicates that the organisms exist abundantly in market broiler chicken from Kampala, with a distribution that is probably dependent on the hygienic practices and storage facilities in processing areas and in the different markets. A big load (>300 cfu) as detected in samples from Local markets, butcheries, and the processing plant could cause infection in human beings. This is a big hazard posed to the consuming public especially where chicken may not be prepared for eating sufficiently to destroy pathogens.

## Recommendations

Raw foods are not sterile, and there are no requirements that they be sterile. So Food processing companies are accountable for following good, up-to-date manufacturing practices that minimise the opportunity of spread of Campylobacter and other bacteria. This can be reached at by institution of GMPs in chicken processing factories. Also freezing should not be relied on to destroy the bacteria. Thorough cooking is what will make the product safe; unless otherwise contaminated by infected raw material.

- A nationwide survey on Campylobacter species in marketchickens should be done to determine the magnitude of the problem and threat to human health.
- Data should also be collected to compare prevalence of other pathogens like Salmonella with Campylobacter in market chicken.
- As a form of risk assessment, diarrhoea cases in hospital should be routinely investigated for Campylobacter species as possible causes and possible sources investigated Public sensitisation about the significance and sources of the organism is paramount.
- A surveillance programme should be initiated based on GMPs compatible with HACCP in processing plants.
- The subsequent studies should focus on what interventions for poultry can be practically achieved and how these intervention impact human health.

## References

*Butzler, J. P. (1984).* 'Campylobacter *infection in Man and Animals'. CRC Press, Boca Raton, FL.*

FDA. (1998) 'Bacteriological Analytical Manual, 8th ed. (Revision A).

Harris, N. V., Kimbal, T., Weiss, N. S. and Nolan, C. M., 1986a 'Diary products, produce and other non-meat foods as possible sources of Campylobacter jejuni and Campylobacer coli enteritis' Journal of Food Protection 49:347-51.

Harris, N.V ., D. Thompson, D. C. Martin, and C. M. Nolan. (1986b). 'A survey of Campylobacter and other bacterial contaminants of pre-market chicken and retail poultry and meats, King country, Washington' Am. J. Publ. Heath 76:401-406.

Hunt, J. M., Abeyta, C., Tran, T. (1998). "Isolation of Campylobacter species from Food and Water". FDA Bacteriological Analytical Manual. Chapt. 7. 8th ed.

ISO: 'Microbiology of food and animal feeding stuffs – Horizontal method for detection of thermotolerant Campylobacter', ISO: 10272 (1995).

MPED: Background to the Budget (1997/1998).

MPED, Background to the Budget (1991/1992).

MPED, Background to the Budget, (1998/1999) 'Budgeting for Poverty Eradication'.

Pitkanen,T. (1982). Ann. Clin. Res. In 'Foodborne bacterial pathogens', by Doyle, P. M., (1989).

Rigney and Associates Researchers' Toolbox: Statistical Tools, 2000. http://www.rigneyassoc.com/samplesize.htm

Sarge Bilgili, (2000). '*Campylobacter* in broilers'. http://www.ag.auburn.edu/dept/ph

Sean F. Alterkruse, Norman J. Stern, Patricia I. Fields, and David L. Swerdlow, (1999). '*Campylobacter jejuni* – An emerging Foodborne Pathogen. Emerging Infectious Diseases'. http://www.cdc.gov/ncidod/EID

Tauxe,R. V., C. M. Patton, P. Edmonds, T. J. Barret, D. J. Brenner, and P. A. Blake. (1985). J. Clin. Microbiology. 21: 222-225.

Tauxe, R. V., Hargrett-Bean, N., Patton, C. M. and Wachsmuth. I. K. (1988) '*Campylobacter* isolates in the United States, *1982-1986*' US Morbidity and Mortality *Weekly Report*: CDC Surveilance Summaries 37:SS-2, 1-13.

WHO: Report of WHO consultation on salmonellosis control in Agriculture. WHO/CDS/VPH/90.94. Orvieto, Italy. Pp. 23.