Prevalence and Risk Factors for *Campylobacter* Infection of Chicken in Peri-Urban Areas of Nairobi, Kenya

**Abstract**

*Campylobacter* species are the most common cause of bacterial food borne disease affecting humans in both developed and developing countries [1]. A cross sectional study was done to determine the prevalence of *campylobacter* in chicken and its associated risk factors in Nairobi between June and December 2015. Fifty six broiler chicken, one fifty four indigenous chicken and sixty two layers were included in the study. Cloacal swabs were obtained from live birds and *campylobacter* status of the birds was determined using culture and multiplex polymerase chain reaction. Data on potential risk factors was collected by administering questionnaires to farmers in farms where cloacal swab samples were obtained. The overall prevalence of *campylobacter* in this study was 69.5% with 91.07% in broiler chicken, 70.96% in layers and 61.04% in indigenous chickens. Approximately seventy seven percent (76.8%) of the isolates from broiler chicken were found to be *C. jejuni* and 14.3% were other *Campylobacter* species. No *campylobacter* isolates from broilers were *C. coli*. Thirty three percent (32.5%) of the isolates from indigenous chicken were *C. jejuni*, 5.84 % were *C. coli* and 15.6% were other *Campylobacter* species while 31.7 % of the isolates from layers were *C. jejuni*, 19.4% were *C. coli* and 9.7% were untypable *campylobacter* species. Logistic regression identified six variables as risk factors for *campylobacter* colonization. They included old age of poultry house (p=0.23), large number of birds kept (p=0.12), increasing age of sale of birds (p=0.01), type of rodent control (p=0.03), inadequate washing and disinfection of poultry house before restocking (p=0.004) and absence of a medicated footbath at the entrance into the poultry house (p=0.05). These findings show that there is a high prevalence of *Campylobacter* infection in all kinds of chicken. The use of disinfected footbath at the entrance to the chicken house, adequate cleaning and disinfection of the chicken house, drinkers and feeders and proper rodent control measures will reduce chances of *campylobacter* colonization in chickens. It was recommended that poultry farmers be educated on various insecurity measures that can reduce *campylobacter* infection in chicken at farm level and the consequences of such infection.

**Introduction**

*Campylobacter* species are the most common cause of bacterial food borne disease affecting humans in both developed and developing countries [1]. It is estimated that 400 to 500 million cases of *Campylobacter* enteritis occur annually around the world [2]. Endocarditis, reactive arthritis, hemolytic uremic syndrome and septicaemia are the complications that are occasionally seen [2,3]. Rare complications such as meningitis, acute cholecystitis and Guillain Barré syndrome have been reported [4,5]. Human infections are mainly caused by *Campylobacter jejuni* and *Campylobacter coli* and rarely by *Campylobacter lari* [5]. There are many possible sources of *campylobacter* infection in humans including contaminated poultry meat [6], untreated raw milk [7] and contaminated water [8-10]. The bacteria in the gastrointestinal tract of chicken and cattle can contaminate the carcass during slaughter and subsequently be transmitted to humans [2]. Milk can be contaminated with *campylobacter* in cattle faeces during milking, while water gets contaminated with chicken or cattle faeces from the environment. The main risk factor for human infection is consumption of raw or undercooked poultry meat contaminated with pathogenic *campylobacter* species [11]. *Campylobacter* species are carried in the intestinal tracts of birds and mammals which shed them in large numbers to contaminate the environment including water sources [12]. Poultry meat is the most commonly incriminated cause of food borne *campylobacter* infections occurring in humans in many parts of the world [2] with fresh chicken as the main risk factor [13]. Avian species especially domestic chickens are frequently infected primarily with *Campylobacter jejuni* and *Campylobacter coli* during rearing [14]. *Campylobacter* invade chicken early in life through contaminated drinking water [15,16], unhygienic chicken house environment [17], wild birds, flies and rodents [18]. Risk factors that have been associated with *Campylobacter* colonization of chickens include season of the year [19], several poultry houses and presence of other animals in the farm [15]. Contaminated drinking water, administration of antibiotics [15,20], poor hygiene [19,21,22], old age of the flock and that of houses at the farm [22] have also been incriminated as risk factors for the infection.

In order to reduce human infection, *campylobacter* should be controlled at the farm level by preventing colonization of chicken gastrointestinal tract and subsequent shedding of the organisms during the rearing period [23]. This can be done by maintaining good hygiene practices and implementation of appropriate biosecurity measures [23,24]. These measures include acidification of drinking water and rodent control...
around the chicken houses [23]. This will reduce contamination of carcasses during slaughter and ensure food safety. Data indicating the prevalence and risk factors associated with campylobacter infections in poultry remains limited in Kenya. The aim of this study was to determine the prevalence of food borne campylobacter pathogens in different chicken production systems in Nairobi’s peri-urban areas and risk factors associated with observed prevalence at farm level. The findings of this study will be used in formulating strategies to control campylobacter infection in poultry.

Materials and Methods

The study was conducted in the peri-urban areas of Nairobi including Ruiru, Kikuyu and Ruai. These areas were selected because there are many poultry farmers who supply chicken and chicken products to the residents of Nairobi and surrounding areas according to Livestock Production officers report (2012).

Study population and sampling design

The target population was all chicken reared in peri-urban areas of Nairobi County between June and December 2015. The study population was chicken reared in Ruai, Ruiri and Kikuyu at the same period of study. A list of farms in the study areas was compiled at the start of the study with details such as the name of the farm and an estimate of the number of birds each farm had. The study farms were randomly selected from this list using randomly generated computer numbers. Data on the number of birds in each farm was used to classify farms into two levels (small and large scale production system). The areas were visited three days a week and during each visit three to four farms were visited for sample collection and administration of questionnaires.

The number sampled in each level was proportional to the total number in the strata.

Questionnaire

A structured questionnaire was administered by the research team to the chicken farmer in each of farms visited during sample collection. The questionnaire had questions concerning: Age of poultry houses, age and number of birds in the houses, season of the year, source of water and water treatment, health status of the flock, type of litter used and bio-security and bio-safety practices at the farm.

Sample collection and submission to the laboratory

A stratified random sampling approach was then used to randomly sample live birds (from farms) for inclusion in the study. The sampling design was to allow for proportional sampling of birds at the individual farm level to ensure that each bird category the farmer had was represented (i.e. broilers, layers, indigenous chickens). Sterile cotton tipped swabs were used to obtain cloacal swabs which were then transported in Stuart’s transport media to the laboratory within three hours after collecting the sample.

Culture and isolation of campylobacter

Sterile cotton tipped swabs with cloacal fecal content were spread onto modified Charcoal Cefoperazone Deoxycholate Agar plates comprising of Campylobacter selective blood free agar base (CM0739, Oxoid, Basingstoke, U.K) and Campylobacter selective supplement (SR0167E, Oxoid, Basingstoke, U.K) containing cefoperazone 1.6mg, vancomycin 10mg, sodium pyruvate 50mg and cycloheximide 50mg as selective supplements. The inoculated plates were incubated at 42°C for 48 hours under microaerophilic conditions created by using candles. After 48 hours, suspect colonies of Campylobacter species were picked with a sterile wire loop and suspended in sterile distilled water in eppendorf tubes for DNA extraction. Campylobacter spp. suspect colonies were identified by colonial morphology, Gram stain, oxidase test and catalase test.

Confirmation and identification of campylobacter species

This was done using a multiplex polymerase chain reaction (PCR) with primers described by Linton et al. [25], Lawson et al. [26] and Bang et al. [27] listed in (Table 1). DNA extraction was carried out using boiling method. A bopulous of suspcet campylobacter colonies was suspended in 500µl of sterile distilled water in an eppendorf tube. It was boiled in a water bath at 100°C for 30 minutes. It was allowed to cool and centrifuged at 15000 rpm for 5 minutes. The supernatant containing DNA was aliquot into a sterile eppendorf tubes for further tests. Multiplex PCR was performed in a total reaction volume of 12.5 µl containing PCR master mix of 6.25µl, and 0.05µl of 0.4M asp-primers CC18F and CCS19R [25], 0.02µl of 0.2M hippurate based and 0.05µl of 0.05M 16S rRNA based primers and 5µl of DNA template. Cycling conditions were initial denaturation at 94°C for 6 min, followed by 35 cycles of denaturation at 94°C for 50s, annealing at 57°C for 40s and extension at 72°C for 50s and final heating 72°C for 5 min. PCR products were analyzed using 1.5% agarose gel electrophoresis and stained with ethidium bromide. Results were documented by photography under UV light. Specific amplification fragments expected were of size 1062bp, 500bp and 344bp corresponded to Campylobacter genus, C. coli and C. jejuni respectively. The unit of observation was the individual bird and each sample represented an individual bird. If campylobacter was detected by PCR in a sample, the bird was considered infected.

Analysis of risk factors

Data from questionnaires was used to determine the most important risk factors associated with campylobacter infection in chicken. This data were entered into Microsoft excel and checked for accuracy before being transferred to STATA Version 7. Logistic regression was used to determine the association between possible risk factors (explanatory variables) and campylobacter status of the flock (outcome variable). Variables with a p-value ≤ 0.25 in the univariate analysis were included in the multivariate logistic regression analysis.

Results

Prevalence of campylobacter infection

Campylobacter was isolated from 189 out of 272 birds sampled, giving a prevalence of 69.5%. The prevalence of campylobacter in broilers was 91.07%, 70.96% in layers and 61.04% in Indigenous...
chicken. Approximately 76.8% of the isolates from broilers were *Campylobacter jejuni* while 14.3% were neither *Campylobacter coli* nor *Campylobacter jejuni*. Of all the *Campylobacter* isolated from layers, 37.1% were *C. jejuni*, 19.35% were *C. coli* and 9.68% were neither *C. jejuni* nor *C. coli*. Approximately 32.5% of the isolates from indigenous chicken were *C. jejuni*, 5.84% were *C. coli* and 15.58% were neither *C. jejuni* nor *C. coli*. *Campylobacter jejuni* was a predominant species of thermophilic *campylobacter* in all categories of chicken. Infection rate in chickens was significantly higher in broilers than layers and indigenous chicken. The results are shown in (Table 2) below. The prevalence of *campylobacter* infection of various chicken types from various study areas is given in (Table 3). The highest prevalence of 91.6% was recorded in Kikuyu, followed by Ruiru with a prevalence of 65.4% and Ruai with a prevalence of 55.9%. The highest prevalence was recorded in layers 93.8%, indigenous chicken 75% and broilers 97.14%, from Kikuyu. The infection rate was lowest for broilers, layers and indigenous chicken from Ruai. Ruiru recorded moderate level of infection in broilers and indigenous chicken.

**Table 1:** Characteristics of primers used in the study.

| Target Species | Primer Code | 5'---------3' Primer Sequence | Amplicon Size(Bp) | Reference |
|----------------|-------------|-------------------------------|------------------|-----------|
| *Campylobacter* spp. | 16S-F 16S-R | GGAGGCAGCAGTAGGGAATA TGACGGGCGGTGAGTACAAG | 1062 | [27] |
| *C. coli* | CC18F CC519R | GGTATGATTTTCTACAAAGCGA ATAAAAGACTATCGTCGCGTG | 500 | [25] |
| *C. jejuni* | hipO-F 16S-R | GACTTCGTGCAGATATGGATGCTT GCTATAACTATCCGAAGAAGCCATCA | 344 | [27] |

**Table 2:** Prevalence of *Campylobacter* infection in various chicken types.

| Species of Chicken | *Campylobacter* Species | No. of Confirmed *Campylobacter* by PCR | Prevalence | 95% Confidence Interval |
|--------------------|-------------------------|----------------------------------------|------------|------------------------|
| Broilers (n=56)    | *Campylobacter* spp     | 51                                     | 91.07%     | 83.6%-98.54%           |
|                    | *Campylobacter* jejuni  | 43                                     | 76.79%     | 65.73%-87.85           |
|                    | *Campylobacter* coli    | 0                                      | 0          | 0                      |
|                    | *C. jejuni and C. coli*| 0                                      | 0          | 0                      |
|                    | Other *campylobacter* species | 8                              | 14.29%     | 5.12%-23.46%           |
| Layers (n=62)      | *Campylobacter* species | 44                                     | 70.96%     | 59.66%-82.26%          |
|                    | *C. jejuni*             | 23                                     | 37.10%     | 25.08%-49.12%          |
|                    | *C. coli*               | 12                                     | 19.35%     | 9.55%-29.15%           |
|                    | *C. jejuni and C. coli*| 3                                      | 4.84%      | -0.106                 |
|                    | Other *Campylobacter* species | 6                              | 9.68%      | 2.38%-16.90%           |
| Indigenous (n=154) | *Campylobacter* species | 94                                     | 61.04%     | 53.34%-68.74%          |
|                    | *C. jejuni*             | 50                                     | 32.47%     | 25.07%-39.87%          |
|                    | *C. coli*               | 9                                      | 5.84%      | 2.14%-9.54%            |
|                    | *C. jejuni and C. coli*| 7                                      | 4.55%      | 1.25%-7.85%            |
|                    | Other *Campylobacter* species | 28                              | 18.18%     | 9.88%-21.28%           |
| Total sample size (n=272) | Total number positive for *Campylobacter* species | 189 | 69.50% | 64%-75% |
Table 3: Prevalence of Campylobacter infection of chicken from different study areas.

| Study Area | Broilers | Layers | Indigenous Chicken | Total |
|------------|----------|--------|-------------------|-------|
|            | No positive (%) | No positive (%) | No positive (%) | No positive (%) |
| Ruai       | 12/15 (80%)    | 14/30 (46.7%)   | 36/66 (54.5%)    | 62/111 (55.9%) |
| Ruiru      | 5/6 (83.3%)    | 0             | 46/72 (63.9%)    | 51/78 (65.4%)  |
| Kikuyu     | 34/35 (97.14%) | 30/32 (93.8%)  | 12/16 (75%)      | 76/83 (91.6%)  |
| Total      | 51/56 (91.07%) | 44/62 (70.97%) | 94/154 (61.04%) | 189/272 (69.5%) |

Identification of risk factors

Twelve variables were tested by univariate analysis and those that had a p value of <0.25 were considered to be significantly associated with Campylobacter infection. Six factors were associated with the infection with a p value less than 0.25 including age of poultry house (p=0.23), number of birds kept (p=0.12), age when birds were sold (p=0.01), type of rodent control (p=0.03), washing and disinfection of poultry house before restocking (p=0.004) and presence of a medicated footbath at the entrance into the poultry house (p=0.05) as shown in (Table 4) below. Almost all the farms had in place bio-security measures. These included washing and disinfection of poultry houses before restocking, keeping birds of different species separately and maintaining the interval between two rearing periods at more than two weeks. Most of the farms had rodents and did not treat drinking water for the birds. The age of birds at the time of sampling was not statistically significant although there was evidence of increased odds of infection with increasing age of birds (OR=1.61). Presence of a medicated footbath at the entrance into the poultry house was highly significant with the risk of infection being 3.44 times higher in farms that lacked a medicated footbath.

Table 4: Descriptive statistics of variables and univariable logistic regression analysis of risk factors for the occurrence of Campylobacter species (p<0.25) in 272 chicken in Nairobi, Kenya.

| Variable                                      | Level          | No Positive (%) | P-Value | Odds Ratio |
|-----------------------------------------------|----------------|-----------------|---------|------------|
| Age of poultry house                          | >3 years       | 106 (56.1)      | 0.23    | 1.68       |
|                                               | <3 years       | 83 (43.9)       |         |            |
| Age of birds                                  | >1 month       | 182 (96.3)      | 0.64    | 1.61       |
|                                               | <1 month       | 7 (3.7)         |         |            |
| Number of birds kept                          | >200           | 43 (22.8)       | 0.12    | 0.4        |
|                                               | <200           | 146 (77.2)      |         |            |
| Chicken mixing with other bird species        | Yes            | 21 (11.1)       | 0       | 0.11       |
|                                               | No             | 168 (88.9)      |         |            |
| Age when birds are sold                       | >1 year (>35 days for broilers) | 121 (64) | 0.01 | 0.32 |
|                                               | <1 year (<35 days for broilers) | 68 (36) | | |
| Cleaning and disinfection of poultry house    | Yes            | 170 (90)        | 0.004   | 0.11       |
|                                               | No             | 19 (10)         |         |            |
| Length of down time                           | >2 weeks       | 121 (64)        | 0.62    | 1.3        |
|                                               | <2 weeks       | 68 (36)         |         |            |
| Presence of rodents                           | Yes            | 144 (76.2)      | 0.99    | 1          |
|                                               | No             | 45 (23.8)       |         |            |
| Type of rodent control                        | Professional   | 17 (9)          | 0.03    | 1.4        |
|                                               | Traps          | 2 (1.1)         |         |            |
|                                               | Keeping cats   | 109 (57.7)      |         |            |
|                                               | No control     | 61 (32.3)       |         |            |
| Presence of medicated footbath at entrance    | Yes            | 49 (26)         | 0.05    | 3.44       |
|                                               | No             | 140 (74)        |         |            |
Previous studies also identified poor quality of drinking water, inadequate house cleaning and disinfection before restocking. Use footbath with disinfectant, old age of poultry house, and Campylobacter jejuni in chicken. This study did not find any. 

This study did not find any. However, Nather et al. [34-38] reported no effect of biosecurity measures and hygiene on the level of Campylobacter infection. The carryover of infection from a previous Campylobacter infected flock to a new flock in the same house is a potential source of Campylobacter infection. This is particularly important in farms where used litter is routinely left in the houses between crops. Prevalence of Campylobacter in chicken in this study was relatively high. The most significant risk factors were increasing age of poultry houses and inadequate cleaning and disinfection of poultry houses before restocking. Controlling Campylobacter infection during rearing could reduce contamination during the later stages of production and ensure food safety. Strict bio-security measures should therefore be put in place to reduce the risk of infection during chicken rearing.

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Multivariable analysis

The six variables that were significant in invariable analysis were screened using multivariable logistic regression analysis in a backward stepwise elimination procedure. Two factors remained in the model including age of poultry house (p=0.071) and house washing and disinfection before restocking (p=0.001) at a significance level of p<0.1.

Discussion

This study reported a Campylobacter prevalence of 69.5% in chicken. The prevalence of Campylobacter in broilers was 91.07%. The prevalence of Campylobacter infection in indigenous birds and layers was 61.04 % and 70.96 % respectively. Campylobacter jejuni was isolated from majority of the samples but few samples also yielded C. coli. A good number of chicken were also infected with other Campylobacter that were not identified. Our findings on the prevalence of Campylobacter are similar to the prevalence of 69.8% reported in chicken in Tanzania by Mdegela et al. [28]. However, it was higher than 51.5% reported from a study done in Kenya on apparently healthy domestic chicken by Turkson et al. [13]. On the other hand, the prevalence in broilers was higher than the 69% reported in Broiler flocks in Tanzania by Mdegela et al.[28]. Poor biosecurity measures including lower frequency of litter turning, inadequate sanitation and hygiene as well as poor spacing in the poultry houses enhances transmission of Campylobacter among broilers. This study also recorded a higher prevalence in indigenous chicken than 50.87% earlier reported in Kenya by Ng’ethe et al. [29], which was however lower than the prevalence of 71% reported in Tanzania [28]. The observed high prevalence of Campylobacter infection of indigenous chicken in this study was attributed to poor management practices as they are perceived to be harder compared to the exotic broilers and layers. A study done on the incidence of Campylobacter in laying hens in Sohag by Hedawey et al. [30] reported an incidence rate of 38%. However, this study found prevalence of 70.96 % among layers. Campylobacter jejuni was the predominant Campylobacter species in broiler chicken with a prevalence of 61.4%. This was consistent with other studies [22,31] but inconsistent with a study done in Thailand by Padungtod et al. [32] where C. coli was reported to be the predominant Campylobacter species in broilers chicken. This study did not find any C. coli in broiler chickens.

This study identified the following risk factors for Campylobacter colonization in chicken. They include failure to use footbath with disinfectant, old age of poultry house, and inadequate house cleaning and disinfection before restocking. Previous studies also identified poor quality of drinking water [9,10], unhygienic conditions of poultry houses [21], presence of other animals such as rodents in the farm [15] as risk factors of Campylobacter colonization. A study done by Humphrey et al. [19] showed a decrease in Campylobacter colonization of chicken with increased use of disinfected footbaths. The frequency of changing the disinfectant in the footbath has an effect on the level of infection. Changing the disinfectant twice weekly reduced the risk of infection in flocks according to an intervention trial done by Gibbens et al. [33]. Weekly change of disinfectant would also reduce the infection risk according to a report by Evans et al. [21]. Old age of poultry house was also a significant factor with houses more than three years having a higher odds of infection than those less than 3 years (p=0.071, OR=1.91). This was possibly due to contamination from previous flocks. Old houses more than three years are associated with poor state of repair and maintenance of the poultry house that encouraged large rodent populations which are reservoirs for Campylobacter infection.

The main risk factor associated with the Campylobacter infection in this study was lack of house cleaning and disinfection before restocking (p=0.001, OR=0.28). Chickens reared in houses that were adequately cleaned and disinfected were 3.6 times less likely to get Campylobacter infection compared to those not adequately cleaned and disinfected. This observation was consistent with the observations made by Evans and Sayers [21] on the role of biosecurity measures and hygiene in reducing Campylobacter infection in poultry. However, Nather et al. [34-38] reported no effect of biosecurity measures and hygiene on the level of Campylobacter infection. The carryover of infection from a previous Campylobacter infected flock to a new flock in the same house is a potential source of Campylobacter infection. This is particularly important in farms where used litter is routinely left in the houses between crops. Prevalence of Campylobacter in chicken in this study was relatively high. The most significant risk factors were increasing age of poultry houses and inadequate cleaning and disinfection of poultry houses before restocking. Controlling Campylobacter infection during rearing could reduce contamination during the later stages of production and ensure food safety. Strict bio-security measures should therefore be put in place to reduce the risk of infection during chicken rearing.

| Source of drinking water for the chicken | Rainwater | 24 (12.7) | Borehole | 77(40.7) | 0.66 | 1.1 |
|------------------------------------------|-----------|-----------|-----------|---------|------|-----|
| Tap water                                | 62 (32.8) |           |           |         |      |     |
| From water vendors                       | 22 (11.6) |           |           |         |      |     |
| River water                              | 4 (2.1)   |           |           |         |      |     |
| Use of treated drinking water            | Yes       | 23 (12.2) |           |         | 0.39 | 0.65|
|                                         | No        | 166 (87.8)|           |         |      |     |
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Conflicts of Interest

None.

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