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Prevalence and risk factors for *Campylobacter* infection of chicken in peri-urban areas of Nairobi, Kenya

Abstract

Campylobacter species are the most common bacterial causes of human gastroenteritis worldwide. 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 37.1% 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.

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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 septicemia are the complications that are occasionally seen.[2,3] Rare complications such as meningitis, acute cholecystitis and Guillain Barré syndrome have been reported[3,4] Human infections are mainly caused by *Campylobacter jejuni* and *Campylobacter coli* and rarely *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 feaces 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.[1,14] *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, Ruiru 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 scale 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 Stuarts 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 Camplobacter selective supplement (SR0167E, Oxoid, Basingstoke, U.K) containing cefoperazone 16mg, vancomycin 10mg, sodium pyruvate 50mg and cycloheximide 50mg as selective supplements. The inoculated plates were incubated at 42°C for 48hours under microaerophilic conditions created by using candles. After 48hours, 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 loopful of suspect *Campylobacter* colonies was suspended in 500µl of sterile distilled water in an eppendrof tube. It was boiled in a water bath at 100°C for 30minutes. It was allowed to cool and centrifuged at 15000rpm for 5minutes. The supernatant containing DNA was aliquot into a sterile eppendrof tubes for further tests. Multiplex PCR was performed in a total reaction volume of 12.5µl containing PCR master mix of 6.25µl, and 0.05µl of 0.4M asp-primers CC18F and CC519R,[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 6min, 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 3 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.

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	GGAGGCAGCAGTAGGGAATA	1062	[27]
	16S-R	TGACGGGCGGTGAGTACAAG		
C.coli	CC18F CC519R	GGTATGATTTCTACAAAGCGA ATAAAAGACTATCGTCGCGTG	500	[25]
C.jejuni	hipO-F	GACTTCGTGCAGATATGGATGCTT	344	[27]
	hipO-R	GCTATAACTATCCGAAGAAGCCATCA		

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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* s 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 chicken75% 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.

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 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%
	Campylobacter species	44	70.96%	59.66%-82.26%
Layers (n=62)	*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%-16.98%
	Campylobacter species	94	61.04%	53.34%-68.74%
Indigenous (n=154)	*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%

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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%)

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	>3years	106(56.1)	0.23	1.68
	<3years	83(43.9)		
Age of birds	>1month	182(96.3)	0.64	1.61
	<1month	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	>1year(>35days for broilers	121(64)	0.01	0.32
	<1year(<35days for broilers)	68(36)		
cleaning and disinfection of poultry house	Yes	170(90)	0.004	0.11
	No	19(10)		
Length of down time	>2weeks	121(64)	0.62	1.3
	<2weeks	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)		
Source of drinking water for the chicken	Rainwater	24(12.7)	0.66	1.1
	Borehole	77(40.7)		
	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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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 hardy 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 3years (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] Annan-Prah A[35] Osano O[36] Huneau-Salaun A et al.[37] Scallan E et al.[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 biosecurity measures should therefore be put in place to reduce the risk of infection during chicken rearing.

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Conflicts of interest

Author declares that there is no conflict of interest.

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