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Incidence of *Campylobacter jejuni* in Poultry Handlers

Sharfadi R.S[1*]., Whong C.M.Z[2]., Kabir, J[3]., Yahaya, H[1]., Ibrahim A[1]., Muhammad, A[1].
[1]Department of Medical Laboratory Science, Faculty of Allied Health Sciences. Bayero University Kano Nigeria
[2]Department of Microbiology, Faculty of Science. Ahmadu Bello University Zaria Nigeria
[3]Department of Veterinary Public Health, Faculty of Veterinary Medicine Ahmadu Bello University Zaria Nigeria

***Corresponding Authors**
Name: Sharfadi RS
Email: rahinatusahmed@yahoo.com

Abstract: Infection with *Campylobacter jejuni* is one of the most common causes of gastroenteritis worldwide. This study is aimed at assessing the incidence of *C. jejuni* in poultry handlers, as a preliminary step of controlling human campylobacteriosis in our study community. Blood samples were collected from one hundred poultry handlers (marketers and processors) in fifteen local live birds markets across the seven local government areas of Kano metropolis. Samples were analysed using *Campylobacter jejuni* IgG ELISA kit. Seventy four percent of the subjects were sero-positive. *Campylobacter jejuni* infection is age and time dependent. Extra caution is therefore necessary in handling live birds to avoid transmission of the disease.
Keywords: Campylobacter jejuni, poultry, gastroenteritis, handlers

INTRODUCTION

Infection with *Campylobacter jejuni* is one of the most common causes of gastroenteritis worldwide [1]; it occurs more frequently than do infections caused by *Salmonella* species, *Shigella* species, or *Escherichia coli* O157:H7 [2]. In developed countries, the incidence of *Campylobacter jejuni* infections peaks during infancy and again during early adulthood [3]. Most infections are acquired by the consumption and handling of poultry [4]. A typical case is characterized by diarrhoea, fever, and abdominal cramps [5]. *C. jejuni* have been isolated from a wide range of animal species and environmental sources [6]. Several studies have shown that eating and handling of improperly cooked or raw poultry meat poses a risk for contracting campylobacteriosis [7,8, 9]. Panel on Biological Hazards estimated that 50 -80% of all campylobacteriosis cases are associated with the chicken reservoir as a whole. The panel has indicated that a reduction in the number of *Campylobacter* spp. positive flocks would be the most cost-effective measure in controlling human campylobacteriosis [10]. Though obtaining cultures of the organism from stool samples remains the best way to diagnose this infection [1], this study is aimed at assessing the incidence of *C. jejuni* in poultry handlers as a preliminary step of controlling human campylobacteriosis in our study community.

MATERIALS AND METHODS

Area of study

This study was carried out in Kano Metropolis, Nigeria; in seven Local Governments namely Dala, Fagge, and Gwale, Kumbotso, Municipal, Nassarawa and Tarauni. Kano Metropolis has a population of about 3,848,885 [11].

Sampling procedure

Fifteen live bird markets were employed in the sample collection. Rijiyar-Lada and Kurnar-Asabe markets (Dala local government), Sabongari market (Fagge local government), Dorayi market (Gwale local government), Sheka, Mariri, Na'ibawa and Zoo road markets (Kumbotso local government). Kurmi, Rimi and Sharada markets (Municipal local government), Yankaba and Kabarin-raka markets (Nassarawa local government) and Tarauni market (Tarauni local government).

Interviewer questionnaire

A structured questionnaire was used to obtain information on poultry such as bird type, source of bird, number of birds sampled and total number of birds per cage. Data of the handlers such as age, duration in contact, history of Gastro intestinal illness, job description marketer/processor or both were obtained.

Blood collection and processing

Before collection of blood samples, we briefed the poultry handlers on what the research was all about and their consent and cooperation was sought. Some of them accepted and others rejected. The blood samples were collected at the convenience of the marketers in the 15 live birds markets. One hundred blood samples from poultry handlers comprising 60 marketers, 36 processors and 4 marketers/ processors. Blood samples from poultry handlers were collected using sterile five milliliters syringe aseptically by venous puncture by a trained laboratory technician. The blood samples taken

were dispensed into plain bottle, transported to the laboratory in ice cooled box. The samples were centrifuged at 3000 rpm for 5 minutes and the sera collected into other clean dry bottles using Pasteur pipettes and stored at -20^{0}C before analysis.

Samples were then analysed using *Campylobacter jejuni* IgG ELISA kit (Serion ELISA Classic *Campylobacter jejuni* IgG. Friedrich-Bergius-Ring 19, Wurzburg, Germany) in accordance with the manufacturer's instructions, in the Department of Microbiology, Ahmadu Bello University, Zaria Nigeria.

Laboratory assay for *Campylobacter jejuni* IgG antibody

Ten micro-litres of the serum sample were diluted in 1000µl buffer with protein and Tween 20. One hundred micro-litre of the diluted sample and100µl of the ready to use control standards (positive and negative controls) were pipetted into the wells. The first well was left empty as blank, the second and third wells served as higher and lower positive controls and the fourth well served as the negative control. The plates were covered with foil paper and mixed thoroughly to prepare a homogenous solution. It was then incubated at 37ºC for 60 minutes in moist chamber. Then it was washed with washing solution i.e. sodium chloride solution with tween 20, 30. The washing was repeated 3 times and excess washing solution was removed by gently tapping the plates on a paper towel. After washing, 100µl of the ready to use conjugate solution i.e. alkaline phosphatase, was pipetted into the wells. Substrate well was left empty.

The micro-titre plates were covered with foil paper and incubated at 37ºC for 60 minutes in moist chamber. Then it was washed with washing solution i.e. sodium chloride solution with tween 20, 30.The washing was repeated 3 times and excess washing solution was removed by gently tapping the plates on a paper towel. After washing 100µl of substrate solution i.e. para nitrophenyl phosphate was pipetted into the wells including the blank well and incubated at 37ºC for 30 minutes in moist chamber. Then 100µl of 1.2N sodium hydroxide stopping solution was pipetted into the wells to stop the reaction and it was read at 405nm with ELISA reader machine (Model 2101, Sigma Diagnostic EIA Multiwellreader. Diversified Equipment Company Inc, 7213 Lockport Place Lorton, VA 22079).

Statistical analysis

The data obtained from this study was subjected to descriptive statistical analysis and presented in forms of tables and figures. Chi square tests was undertaken to determine if there was association between the Elisa results and the variables of age, duration in contact with poultry, job description and history of gastro-intestinal illness. Values were significant when $p < 0.05$.

RESULTS

ELISA results for the poultry handlers across Kano metropolis were presented in Table 1. Sixty two handlers were strongly infected, twelve exhibited weak infection, and one subject was on the border line while twenty five were not infected. Sixty poultry marketers, thirty six processors and four marketers/processors in Kano metropolis were sampled across the seven local government areas (LGA_S) in Kano metropolis; Dala LGA (7 marketers and 8 processors), Fagge LGA (5 marketers, 6 processors and 1marketer/processor), Gwale LGA (1marketer and1marketer/processor), Kumbotso LGA (18 marketers, 8 processors, and 1marketer/processor), Municipal LGA (24 marketers and 7 processors), Nassarawa LGA (4 marketers, 5 processors and 1marketer/processor) and Tarauni LGA (1 marketer and 2 processors) as shown in Table 2. From the result, it appears that Kumbotso and Municipal LGAs had higher marketers while Dala and Kumbotso LGAs had higher processors.

Table 3 presents the distribution of poultry handlers according to age group. In the age group 19 or below; 15 poultry handlers were sampled out of which 7 (46.7%) were positive with *C. jujeni* infection while 8 (53.3%) were negative. Within the age group 20-39, 56 poultry workers were sampled, 44 (78.6%) and 12 (21.4%) were negative. Twenty seven poultry workers aged 40-59 years were sampled, 21(77.8%) were positive and 6 (22.2%) were negative. Two poultry handlers within age group sixty and above were all positive.

Table 4 presents distribution of poultry handlers according to age. From the result, 35 marketers and 17 processors within the 20-39 year group show higher prevalence. Duration of poultry handlers in contact with the infection within 5-9 and 10 and above indicated higher prevalence.

The history of gastro intestinal tract (GIT) illness in poultry handlers was presented in Table 6. Poultry handlers with the history of GIT for the period of 12 months and above showed higher prevalence (20). However, the condition of 53 of the poultry workers was not determined.

Table-1: ELISA result of the poultry handlers based on their level of reaction

Degree of reaction	Frequency
Strongly Positive	62
Weakly Positive	12
Borderline	1
Negative	25
Total	100

Table-2: Job description of the poultry handlers in local governments of Kano metropolis

	Job description			
LGAs	**Marketer**	**Marketer & Processor**	**Processor**	**Total**
Dala	7	0	8	0
Fagge	5	1	6	12
Gwale	1	1	0	2
Kumbotso	18	1	8	27
Municipal	24	0	7	31
Nassarawa	4	1	5	10
Tarauni	1	0	2	3
Total	60	4	36	100

Table-3: Age group distribution of the poultry handlers sampled in live bird markets

Age group	Frequency	Positive	Negative
19 or <	15	7 (46.7%)	8 (53.3%)
20 – 39	56	44 (78.6%)	12 (21.4%)
40 – 59	27	21 (77.8%)	6 (22.2%)
60 - >	2	2 (100%)	Nil
Total	100		

Table-4: Job description of the poultry handlers in relation to their age group

	Job description			
Age (yrs)	Marketer	Marketer & Processor	Processor	Total
19 or <	5	0	10	15
20 – 39	35	4	17	56
40 -59	20	0	7	27
60 or >	0	0	2	2
Total	60	4	36	100

Table-5: Duration of the poultry handlers in contact with chickens

Duration in contact with birds	Frequency
(yrs)	
1 or <	15
1-4	8
5-9	55
10 or >	22
Total	100

Table-6: History of gastrointestinal illness of the poultry handlers and their job categories

	History of GIT illness (months)					
Job description	3 or <	4-6	7-9	10-12	12	ND
Marketer	8	4	1	1	11	
Marketer/processor	1	0	0	0	1	
Processor	8	3	1	0	8	
Total	17	7	2	1	20	53

Key: ND = Not Determined

DISCUSSION

Campylobacter spp. is recognized worldwide as the leading cause of zoonotic enteric human infections in most developed and developing countries [12]. Our study revealed seventy four percent of poultry handlers to be infected with *C. jejuni.* The main source of human *Campylobacter* infection is believed to be poultry meat based [13,14] and the fact that case-control studies conducted worldwide repeatedly have identified handling of raw poultry and eating poultry products as important risk factors for sporadic Campylobacteriosis [15]. Contamination can also occur indirectly through the hands of the processors and material or instrument used in processing [16].

Our study indicated high incidence of *C. jejuni* infection among handlers in Kano metropolis; a particularly important geographical zone for animal production and animal product processing. Consumer exposure to *Campylobacter* is unavoidable if chicken meat is not handled hygienically, that is by cross contamination from raw poultry meat to ready-to-eat food and/or if the meat is not properly cooked before consumption [14,17].The investigation of Sera from poultry handlers in live birds markets of Kano metropolis clearly demonstrates that many of these individuals have circulating IgG antibodies, detectable by ELISA directed against *Campylobacter* antigens. Occupational exposure of susceptible humans to *C. jejuni* appears to result in resistance to disease.

Previous reports indicated that most individuals develop circulating antibody responses following infection with *Campylobacter jejuni* [18,19]. This is believed to be due to acquired protective immunity [20]. In our study, the levels of such antibodies appeared to be directly related to the duration of endemic exposure. The populations in contact with birds for ten years and above have high positivity rates. It is generally assumed that these acquired antibody responses are concomitant with the induction of protective immunity. This immunity protects individuals from overt disease but not necessarily from colonization. Age related changes in the prevalence of *Campylobacter* infection are well documented [21], but indicated a peak rise in young adults 20- 29 years [22]. This statement is in agreement with our finding in this work. The age group 20 – 39 years has the highest population with raised IgG levels.

C. jejuni infection is anecdotal among poultry abattoir workers that during the first period of employment they suffer from episodes of diarrhoea. However over time the number of diarrhoeal episodes decreases, suggesting acquired immune protection. This is clearly indicated by the frequency (17%) of subjects that had a history of GIT for 3 months or below and this declined to 7% in 4 - 6 months and to 2% in 7 - 9 months and finally to 1% in 10 - 12 months. However those that had history of GIT illness in 12 months and above seem to be on the contrary.

CONCLUSION AND RECOMMENDATION

C. jejuni infection is evident in our study community; proper hygienic practices are therefore needed to prevent transmission of the infection.

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