

Surveillance for *Campylobacter* infections in indigenous poultry reared in Nsukka, Nigeria

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Abstract

Campylobacter is the leading cause of bacterial foodborne diseases worldwide. Poultry is indisputably the major reservoir of *Campylobacter* for human infection but dearth of data exists on the epidemiology of the infection in poultry in Nigeria. Consequently, the study determined the occurrence and distribution of *Campylobacter* infections and also the risk practices aiding the transmission in indigenous poultry reared in Nsukka, Nigeria. The bacteria isolation was done following standard microbiological protocol. The overall prevalence of the infection was 18.9%. The specific prevalence was 19.6% and 17.8% in indigenous chicken and turkey respectively. In the chicken, frizzled feather and naked neck breeds had prevalence of 30.8% and 26.1% respectively while the normal feather breed had the lowest prevalence of 13.7%. In both chicken and turkey, the infection was more during the wet season and in birds raised under extensive husbandry management. Major risk practices found were sale of untreated poultry faeces as organic fertilizer, eating during farm operations and non-use of protective clothing. The 18.9% prevalence is lower than 36% reported in 2010 but is very significant from food safety and public health perspectives. Considering the zoonotic and economic consequences associated with *Campylobacter* infection, adoption of farm-to-fork concept principles in indigenous poultry production in Nsukka is recommended, for further reduction or possible elimination of the infection. Proper treatment of poultry faeces before disposal or before use as feed (in fish or pig farms) or manure (in vegetable gardens) is important to curtail inter species transmission of *Campylobacter* in the study area.

Keywords: *Campylobacter*; food safety; indigenous poultry; local chicken; Nigeria; public health; risk practices

Introduction

Domestication of different species of poultry for meat or egg production is perhaps one of the oldest agribusinesses in human history. With an estimated poultry population of about 180 million as at 2015, producing 454 billion tons of poultry meat and 3.8 million eggs annually; Nigeria is the second largest producer of poultry in Africa (FAOSTAT, 2018). These data may have increased tremendously due to recent increase in poultry production activities in the country, particularly in the Southeast. The poultry subsector is the most commercialized agricultural subsector and has been adjudged the most dynamic and the fastest growing

agribusiness in Nigeria; contributing about 10% of the agricultural gross domestic product (Heise *et al.*, 2015; World Bank, 2017). Consequently, poultry production has significantly contributed to job creation, poverty alleviation, crime reduction and production of the much-needed animal protein in the country (Njoga *et al.*, 2019).

The Nigerian indigenous poultry (NIP), made up of the Nigerian indigenous chicken (NIC) and Nigerian indigenous turkey (NIT) are members of the *Gallus gallus domesticus*. The species have continued to play crucial roles in the boom being experienced in the poultry subsector and in the economic wellbeing of the farmers. The NIC alone constitute about 60% of the poultry population in Nigeria and majority of these are reared under the extensive or scavenging free range production system in rural household (Yakubu *et al.*, 2009; Ogie *et al.*, 2013). With over 13 million Nigerian rural households involved in the production of NIP (World Bank, 2017), these poultry species have not only supported the economy of these families but have also augmented the supply of animal proteins, which unfortunately lags behind the supply in Nigeria (Ajibo *et al.*, 2020), with over 203 million people.

Unfortunately, most avian species including the NIP are highly susceptible to *Campylobacter* infection, particularly *C. jejuni* and *C. coli*, popularly known as the thermophilic or thermotolerant species. *Campylobacters* are fastidious, spiral or rod-shaped Gram-negative bacteria infecting all warm-blooded animals. The organisms are flagellated (unipolar or bipolar) and are approximately 0.2 to 0.8 by 0.5 to 5 μm in dimension (Kaakoush *et al.*, 2015). Most *Campylobacter* species do not produce spores but grow under microaerophilic conditions (5% O₂, 10% CO₂ and 85% N₂) at 42 ± 1 °C (Nwankwo *et al.*, 2016). Of the 28 validly described species and subspecies of *Campylobacter*, the thermophilic *Campylobacter* species (TCS) are the most important members of the genus (Modi *et al.*, 2015), responsible for about 98% of all cases of campylobacteriosis in humans and animals (Gahamanyi *et al.*, 2020).

The susceptibility of poultry to *Campylobacter* infection or colonization, more than other food-producing animals (Sharp, 2016), may be due to the similitude in the temperature-growth requirement of the bacteria (42 ± 1 °C) and that of the physiological temperature of the avian species (42 ± 1 °C). As a result, poultry are the major reservoirs of *Campylobacter* organisms, for transmission to other food-producing animal or humans via the food chain (Sharp, 2016). The TCS, especially *Campylobacter jejuni*, are highly infective; such that just about 40 colony-forming unit (CFU) of the organism can produce disease in human and animal host (Newell and Fearnley, 2003).

Cases of foodborne illnesses due to *Campylobacter* in developed and developing countries have superseded that caused by classical enteric bacteria such as *Salmonella* and *Escherichia coli* (Facciola *et al.*, 2017). Transmission of TCS proceeds essentially through the feco-oral route in both humans and animals. Globally, food safety concerns for transmission of zoonotic microbial pathogens via consumption of foods of animal origin have grown significantly in recent times, due to increased frequency of isolation of these pathogens in food-producing animals (Kaakoush *et al.*, 2015). Humans usually acquired the infection via consumption of infected raw or undercooked foods of animal origin (Torralbo *et al.*, 2014). Additionally, unhygienic food preparation practices, contamination of ready-to-eat foods, drinking of contaminated water and swimming in natural water bodies are major means of propagating *Campylobacter* infections in humans (Ugboma *et al.*, 2013; Torralbo *et al.*, 2014; Szczepanska *et al.*, 2017).

Animal-human cohabitation, that still subsists in rural settings (Onunkwo *et al.*, 2018), and none use of personal protective equipment (PPE) among occupationally exposed individuals (animal health workers, poultry farmers, live bird sellers and poultry carcass processors) exacerbate inter-species transmission of *Campylobacter* at the poultry-human interface (Ekere *et al.*, 2018; Njoga *et al.*, 2019). Moreover, the infection can still spread via contact with infected pet. In Denmark, *Campylobacter jejuni* was isolated from a woman living in the same household with an infected dog (Damborg *et al.*, 2004). Evidence of the zoonotic

transmission was further confirmed on detection of quinolone-resistance genes, with $\geq 95\%$ similarity, from both the dog and human isolates (Damborg *et al.*, 2004).

Immunologically naive or compromised individuals (pregnant women, the elderly, children less than 5 years, patients on immune suppressing drugs) are at higher risk of *Campylobacter* infection. Human campylobacteriosis is characterized by nausea, vomiting, diarrhea, dysentery, headache, fever, abdominal cramps and myalgia (Coker *et al.*, 2003; Giwimi *et al.*, 2015; Modi *et al.*, 2015). These symptoms may develop 1-10 days post infection depending on the health and immune status of the individual. Although mortality rate associated with the human infection is low as some cases usually do not require antibiotic therapy (Hlashwayo *et al.*, 2020), the organisms may cause severe health complications such as Guillain-Barré syndrome, reactive arthritis, irritable bowel syndrome and post-infectious polyneuropathy that may lead to paralysis (Ajene *et al.*, 2013; Esan *et al.*, 2017; Szczepanska *et al.*, 2017).

In animals, *Campylobacter* infections rarely result in clinical disease in poultry but the infected birds remain sources of the infection to humans or other animals reared in the same environment. In ruminants however, *Campylobacter* causes infectious/septic abortions, infertility problems and other economically important health conditions (Sahin *et al.*, 2017). Infection with *C. jejuni* caused massive abortions in sheep in the US and molecular evidence later proved that the infection was due to zoonotic transmission of an emergent highly pathogenic clone of the organism (Sahin *et al.*, 2012).

In view of the zoonotic and economic importance of *Campylobacter* and the role of poultry in transmission of the infection, there is need for active surveillance for the infection especially in NIP reared in close proximity with humans and other food animals. Despite the importance, epidemiological data on the occurrence, distribution and transmission dynamics of TCS in NIP are sparse or where available far-between. Therefore, the study determined the occurrence and distribution of *Campylobacter* infections and also the risk practices aiding the transmission in indigenous poultry reared in Nsukka, Nigeria; to highlight the present status of the infection and recommend appropriate public health action.

Materials and Methods

Study location, study design and data collection

The demographics and geographical location of the study area, Nsukka agricultural zone, has been described (Abonyi and Njoga, 2020). Cross-sectional study design involving *Campylobacter* isolation from faecal samples and interview schedule was adopted in the study. A minimum sample size of 276 birds was calculated for the study, as described by Pourhoseingholi *et al.* (2013) based on 36% prevalence reported by Akwuobu *et al.* (2010). However, for accuracy and buoyancy of data, a total of 316 faecal samples (209 from chicken and 107 from turkey) were randomly and aseptically collected for isolation of TCS. The faecal samples were collected directly from the cloacae, with the aid of a sterile swab sticks. For each sampled bird, the species, breed, sex, husbandry management system and season of the year were noted and recorded. Thereafter, the samples were transported in cold condition to the laboratory for the bacteria isolation. Interview schedule was used to obtain information on involvement of the poultry farmers in risk practices aiding *Campylobacter* infection and transmission.

Isolation of thermophilic Campylobacter species

The *Campylobacter* isolation was done using modified charcoal cefoperazone deoxycholate agar (MCCDA, CMO739, Oxoid, UK). The MCCDA was prepared according to the manufacturer's instruction and supplemented with a *Campylobacter* supplement (SRO 155E, Oxoid, UK) to aid the bacteria isolation.

The swab samples were streaked directly on the supplemented agar and the set up incubated under microaerophilic conditions, generated by CampyGen® (CN 0025A, Oxoid, UK) at 42 °C for 48-72 hours.

In plates that yielded growth, the suspected bacteria colonies appearing flat, glossy and thinly spreading on the agar surface were purified on supplemented MCCDA under the same incubation conditions. Subsequently, putative pure colonies were subjected to biochemical characterization according to the methods of Barrett *et al.* (1988). Colonies that tested positive to biochemical characterization were Gram-stained and viewed under the oil-immersion objective. Presence of Gram-negative short rods or curved/spirally-shaped organisms were confirmatory.

Statistical analyses

Fishers' exact test was performed to check for significant statistical association ($p < 0.05$) between the bacteria infection and the poultry species and breeds. The same statistic was used to test for significant association ($p < 0.05$) between TCI and epidemiological variables. The tests were performed at 5% probability level using GraphPad Prism® version 6.04 (GraphPad® Inc., San Diego, California, USA). Significance was accepted at $p < 0.05$.

Results

The overall prevalence of TCI in NIP was 18.9% (60/316). Specifically, the prevalence of the infection in NIC was 19.6% (41/209) while that of NIT was 17.8% (19/107) as shown in Table 1. No statistical significance ($p > 0.05$) was noted between the bacteria infection and the poultry species even though the infection was slightly higher in the chicken than in the turkey (Table 1)

Results on the breed distribution of *Campylobacter* infections in indigenous chicken reared in Nsukka agricultural zone are presented in Table 2. The frizzled feather and naked neck breeds had prevalence of 30.8% and 26.1% respectively while the normal feather breed had the lowest prevalence of 13.7%. Statistical significance ($p < 0.05$) was found between the occurrence of the infection and the breeds (Table 2).

Similarly, findings on the distribution of *Campylobacter* infections in NIC and NIP according to various epidemiological variables are shown in Tables 3 and 4 respectively. In the NIC, there was preponderance of the infection during the wet (rainy) season (24.3%) and in those raised under extensive management system (28.4%). There was significant association ($p < 0.05$) between TCI and husbandry management system but no significant association ($p > 0.05$) existed between the infection and sex or season (Table 3).

In turkey, the infection also predominated during the wet season (20.7%) and in extensively raised turkeys (23.7%). However, no significant association ($p > 0.05$) occurred between the infection and any of the epidemiological variables - sex, season and husbandry production system (Table 4).

Major risk practices aiding *Campylobacter* infection and spread in the farms/households surveyed were sale or use of untreated poultry faeces as organic fertilizer, eating during farm operations and non-use of PPE while on routine farm duties. In farms that practiced intensive husbandry system, risk practices found were non-treating or sanitization of drinking water, non-disinfection of feeding and drinking troughs and non-isolation of exposed birds or those returned unsold from the market.

Table 1. Prevalence of thermophilic *Campylobacter* infections in indigenous poultry (n=316) reared in Nsukka, Nigeria

Poultry species	Number tested	Number infected	Prevalence	Odds ratio	95% CI	P-value
Chicken	209	41	19.6	1.1	0.62 - 2.2	0.763
Turkey	107	19	17.8			

Total	316	60	18.9			
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Fishers' exact test, GraphPad Prism 6.04; CI = Confidence interval

Table 2. Breed distribution of *Campylobacter* infections in indigenous chicken (n=209) reared in Nsukka, Nigeria

Indigenous chicken breeds	Number tested	Number infected	Prevalence	P-value
Naked neck	46	12	26.1	0.029*
Fizzled feather	39	12	30.8	
Normal feather	124	17	13.7	
Total	209	41	19.6	

*Denotes statistical significance, Fishers' exact test, GraphPad Prism 6.04

Table 3. Distribution of *Campylobacter* infections in Nigerian indigenous chicken (N=209) according to various epidemiological variables

Epidemiological variables	Number tested	Number infected	Prevalence	Odds ratio	95% CI	P-value
SEX						
Male	73	14	19.1	0.96	0.47 - 2.1	0.998
Female	136	27	19.9			
SEASON						
Wet	107	26	24.3	1.9	0.27 - 1.1	0.085
Dry	102	15	14.7			
HUSBANDRY						
Extensive	88	25	28.4	2.6	0.19 - 0.77	0.008*
Intensive	121	16	13.2			

*Denotes statistical significance, Fishers' exact test, GraphPad Prism 6.04; CI = Confidence interval

Table 4. Distribution of *Campylobacter* infections in Nigerian indigenous turkey (n=107) according to various epidemiological variables

Epidemiological variables	Number tested	Number infected	Prevalence	Odds ratio	95% CI	P-value
SEX						
Male	44	8	18.2	1.1	0.39 - 2.9	0.996
Female	63	11	17.5			
SEASON						
Wet	58	12	20.7	0.64	0.28 - 1.5	0.393
Dry	49	7	14.3			
HUSBANDRY						
Extensive	38	9	23.7	1.8	0.67 - 5.0	0.298
Intensive	69	10	14.5			

Fishers' exact test, GraphPad Prism 6.04; CI = Confidence interval

Discussion

The overall prevalence of 18.9% as well as the specific prevalence of 19.6% and 17.8% recorded for indigenous chicken and turkey respectively are lower than 36% reported in the same study area by Akwuoba *et al.* (2010). This is a clear indication that *Campylobacter* infection in indigenous poultry is decreasing in Nsukka, Nigeria. The decline may be due to intensive husbandry system of poultry production adopted in some farms surveyed. Being research farms, some of these farms practiced intensive husbandry system which may have reduced microbial infections in the animals. This husbandry system may have also limited exposure of the birds

to harsh climatic conditions which facilitate infection with pathogens, especially in the tropics (Njoga *et al.*, 2018). The husbandry system also limits incursion of wild or migratory birds, which are known natural reservoirs of pathogenic *Campylobacter* species (Dasti *et al.*, 2010; Szczepanska *et al.*, 2017), hence the low prevalence.

Moreover, prophylactic administration of antimicrobial agents widely practiced in poultry farms nationwide (Njoga *et al.*, 2018) may have also contributed to the reduction in *Campylobacter* infection. Although TCI do not usually result in clinical disease in poultry, prophylactic administration of antimicrobial agents for management of other economically important bacterial diseases may have depopulated even the non-targeted organisms such as *Campylobacter*. The resultant effect therefore may have been decrease in *Campylobacter* colonization of poultry and thus the low prevalence being reported.

The overall and species-specific prevalence found in this work are also lower than 38.8% reported in indigenous chicken in Sokoto State, Northwest Nigeria (Salihu *et al.*, 2009). The dissimilarities in the findings could be attributed to dichotomies in environmental, epidemiological or climatic factors at the study locations, which may have influenced the research outcomes. These factors include husbandry management systems, seasons of the year, health status of the birds and discrepancies in researchers' expertise in *Campylobacter* isolation and characterization.

Although the 18.9% prevalence being reported has been adjudged low, the prevalence of the infection was not expected to be that much considering the unique resilience and resistance of NIP to microbial infections (Ogie *et al.*, 2013). The infection was this much probably due to poor biosecurity practices and the scavenging feeding habits of indigenous poultry, especially those reared under extensive production system. These afford poultry unrestricted access to wild and migratory birds which are natural reservoirs of *Campylobacter* (Dasti *et al.*, 2010).

The 19.6% and 17.8% prevalence reported for indigenous chicken and turkey respectively may be lower than earlier reports (Salihu *et al.*, 2009; Akwuobu *et al.*, 2010; Nwankwo *et al.*, 2016) but they are very significant from food safety and public health perspectives. Infection of one in every five-chicken surveyed (i.e. 19.6%) portends great public health risk in the study area due to variety of reasons. Many people are engaged in poultry production in Nigeria and majority of these people do not use PPE during routine duties (Njoga *et al.*, 2019). Poultry and poultry products enjoy wide acceptance across diverse ethnic backgrounds and religious beliefs. For instance, people relish poultry eggs (as the cheapest source of animal protein with 100% biological value) and chicken due to its low cholesterol content. Moreover, the NIC are usually reared organically such that the products contain no or little antimicrobial drug residues. These reasons make the risk of the human infection via the food chain very high.

Considering the role of poultry and poultry products in human campylobacteriosis (Sharp, 2016) and the ease of contamination of poultry meats during the carcass processing; human *Campylobacter* infection could become a public health emergency in the study area. However, the health emergency may not arise easily as most Africans have the culture of proper meat cooking in which meats are cooked (boiled, fried or grilled) at temperature range of 60 to 90 °C for about 30 minutes (Okoli *et al.*, 2018). This type of cooking ensures that most meat bacterial contaminants are killed-off or inactivated, thereby limiting the chances of the transmission through the food chain.

Beyond the human health problems, a major significance of the findings of this work is that the 19.6% infected chicken could serve as reservoirs of *Campylobacter* for infection of other food animals; especially small ruminants usually raised in close proximity with poultry. Abortion storms and other devastating disease conditions in ruminants due to *Campylobacter* infection has been reported (Sahin *et al.*, 2012; 2017). There is also the possibility of contaminating drinking water sources with these enteric pathogens during disposal of poultry offal's or wastes from infected chicken; since slaughterhouse wastes or effluents are usually washed into natural water bodies during the rainy season (Njoga *et al.*, 2019). *Campylobacter* organisms have been isolated

in ground water (Ugboma *et al.*, 2013). Drinking of such contaminated water has been associated with onset of diarrhea, especially in children.

Apart from aiding the contamination of natural water bodies which sometimes are sources of drinking water in rural settings, the role of rain and rainy (wet) season in the epidemiology of *Campylobacter* infection may be significant. It seems that moisture conditions associated with rainy season facilitates *Campylobacter* survival and transmission. This probably explains the preponderance of the infection during the rainy season in both the chicken and turkey species as found in this work. The seasonality in the infection as found in this research is in tandem with the findings of other researchers outside the study area (Nwankwo *et al.*, 2018; Djennad *et al.*, 2019) and this finding could be exploited in the control of the infection.

Conclusions

Since poultry is the major source of human *Campylobacter* infection and human health is complicatedly connected to that of animals, effort towards further reduction or possible elimination of *Campylobacter* infection in poultry is worthwhile. Good farm management practices and adoption of farm-to-fork concept of food safety in production of NIP in Nsukka are crucial in this regard. Proper treatment of poultry faeces before disposal or use as feed (in pig and fish farms) or manure (in vegetable and fruit gardens), are advocated to further reduce the transmission of *Campylobacter* and the associated health and economic problems.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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