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Epidemiology of thermotolerant *Campylobacter* infection in poultry in Nsukka agricultural zone, Nigeria

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Abstract

Background and Aim: Thermotolerant *Campylobacter* organisms (TCOs) are primary causes of bacterial foodborne gastroenteritis worldwide. Although all warm-blooded animals are susceptible to colonization by TCOs, food-producing animals, especially poultry, are major reservoirs of the infection for transmission to humans. This epidemiological study for thermotolerant *Campylobacter* infection (TCI) in poultry was, therefore, conducted to determine the prevalence and to identify the risk factors of TCI in 60 randomly selected poultry farms in Nsukka agricultural zone.

Materials and Methods: A structured questionnaire was used to elicit information on the farmers' involvement in practices that may aggravate TCI in poultry farms. Isolation of TCOs for the determination of prevalence of the infection was done following standard microbiological protocol.

Results: The majority (93.3%) of the farms practiced intensive management system. Farm and individual prevalence of TCI were 78.3% and 19.4%, respectively. The prevalence of 15.7% and 23.6% was recorded for birds reared in urban and rural areas, respectively. Similarly, prevalence rates of 17.2%, 25%, 14.7%, and 24.5% were documented for broiler, layer, male, and female birds, respectively. Major risk factors of TCI found were non-sanitization of drinking water, rearing birds of different ages together, thinning, raising other animals alongside poultry, and overstocking.

Conclusion: Overall prevalence of 19.4% is high from public health and food safety points of view. Farmers' participation in the risk factors for TCI is massive. Significant improvement in biosecurity practices in poultry farms in the study area is therefore imperative; to limit TCI in poultry and hence the risk of human infection through the food chain or at the poultry-human interface.

Keywords: biosecurity, Nigeria, poultry, public health, risk factors, thermotolerant *Campylobacter* infection.

Introduction

Thermotolerant *Campylobacter* organisms (TCOs) are major threats to public health, being the predominant causes of bacteria foodborne illnesses in both developed [1] and developing [2] countries. The organisms are Gram-negative bacteria belonging to the family *Campylobacteraceae* and genus *Campylobacter* [3]. *Campylobacters* are small (0.2-0.9 µm wide and 0.5-6.0 µm long) and rod shaped [4], although coccoid or spherical-shaped forms are usually found in old or stress-exposed cultures, as viable but non-culturable cells. The TCOs, especially *Campylobacter jejuni*, are highly infective, requiring just about 500 cells to produce disease in humans [5].

Although members of the genus *Campylobacter* consist of 32 validly described species and 13 subspecies [4-6], the thermophilic species are the most important members of the genus from food safety and public health points of view. Their complicity in colonization of food-producing animals at farms and ease of transmission of the pathogens to humans through the food chain [7,8] makes the organisms essential zoonotic agents. Although, all food animals can be reservoirs of thermotolerant *Campylobacter* infection (TCI), poultry are the undisputable major reservoirs of the organisms, due to heavy colonization of their gastrointestinal tracts (GIT) early in life through horizontal transmission [6,9]. The predilection of TCOs for poultry GIT may be due to the similarity in the temperature growth requirement of the organisms (42±1°C) and the physiological temperature of poultry (42±1°C).

Transmission of TCOs in both animals and humans proceeds essentially through the feco-oral route. Humans usually acquired the infection through consumption of infected raw or undercooked foods of animal origin, especially poultry [10,11]. In addition, unhygienic food preparation practices and contamination of ready-to-eat foods with raw chicken are major sources of the infection and means of transmission of *Campylobacter* in humans [3,10,11]. Moreover, close contact with infected birds may facilitate the

spread of the infection, especially among occupationally exposed (poultry farmers, live bird sellers, and poultry carcass processors) and immunologically naive or compromised (pregnant women and children under 5 years of age) individuals [12]. Extensive husbandry systems of food animal production and animal-human cohabitation in rural settings enhance intra- and inter-species transmission of *Campylobacter* organisms [13,14].

Human campylobacteriosis develops approximately 1-5 days post-infection [1,15] and is characterized by gastrointestinal problems such as nausea, vomiting, diarrhea, dysentery, headache, fever, abdominal cramps, and myalgia. Although, mortality rate associated with campylobacteriosis is low and infections may resolve without antibiotic treatment [8,16], the organisms cause severe debilitating illnesses and infrequent fatal complications in humans, such as Guillain-Barré syndrome, reactive arthritis, Reiter's syndrome, irritable bowel syndrome, inflammatory bowel disease, Crohn's disease, ulcerative colitis, hemolytic uremic syndrome, and meningitis [17,18].

Food safety concerns for campylobacteriosis have grown rapidly due to frequent isolation of antimicrobial-resistant thermophilic *Campylobacter* strains in food-producing animals, especially poultry [3,8,19,20]; and the ease of transmission of this zoonosis and/or the antimicrobial-resistant organisms through consumption of the contaminated food or water [21]. The high rate of emergence and dissemination of antimicrobial-resistant *Campylobacter* organisms is due to large-scale indiscriminate use of antimicrobials in animal agriculture and antibiotic abuse in humans [22,23]. Consequently, edible tissues from food animals may become vehicles for the transmission of antimicrobial-resistant *Campylobacter* strains to humans through the consumption of the undercooked tissues [3,7].

The epidemiology of TCI in poultry in Nsukka agricultural zone is not well understood due to dearth of data on the prevalence and risk factors of the infection. Research findings elsewhere [11,24] indicated that the age of birds at slaughter, rearing of other animals within poultry farm premises and heavy rainfall shortly before slaughter are risk factors for *Campylobacter* infection in poultry. However, it is not clear whether these factors apply in the study area. Furthermore, the specific role or contribution of poultry to the overall *Campylobacter* burden has not been elucidated in the study area, despite large-scale production and consumption of poultry and poultry products.

Despite a few reports on *Campylobacter* in some parts of the country [25-27], campylobacteriosis has remained a neglected zoonosis [28] due to a lack of epidemiological information on the transmission dynamics. Furthermore, there is no existing national surveillance program or database information relating to *Campylobacter* infections in food animals in Nigeria.

This study determined the prevalence and risk factors for TCI in poultry. This will highlight the current status of the infection and aid a better understanding of *Campylobacter* transmission mechanism in Nsukka agricultural zone. The study may also guide policy formulation in the selection of appropriate control measures and institutions of public health action against TCI in view of the health and economic consequences thereof.

Materials and Methods

Ethical approval

Ethical approval is not applicable to this study as only stool samples from poultry were used in this study.

Informed consent

Oral consent to partake in the study was requested and obtained from poultry farmers included in this study.

Study location

The study was carried out in Nsukka agricultural zone of Enugu State, Southeast, Nigeria. The zone is the largest of the three agricultural zones of the state, with map coordinates of 6°51′24″N and 7°23′45″E [29]. The study location has a total land area of about 5545.38 km^2 and a population of about 2.5 million people [22,23]. Nsukka is characterized by two tropical climatic seasons: Wet/rainy (winter) and dry/hot (summer); low relative humidity of 14%, an annual mean rainfall range of 168-170 mm, and preponderance of Guinea savannah vegetation [30]. Although the majority of Nsukka citizenry are involved in crop farming and civil service, subsistent food animal production, especially poultry farming, is extensively practiced, to augment income or as precaution against crop yield failure.

Study design

The study adopted a cross-sectional study design, consisting of two phases. The first phase involved the use of a structured questionnaire to obtain information bordering on sociodemographics of the farmers, biosecurity practices adopted in poultry farms, and the farmers' involvement in practices that exacerbate colonization and transmission of TCI in poultry. Sixty poultry farmers, one per farm, who consented to partake in the study, and were either the farm owners or manager, were randomly selected and surveyed. The second phase was isolation of TCOs, according to standard microbiological method, to determine the prevalence of TCI in poultry and poultry farms in the study area.

Sample collection

Although 299 fecal samples were calculated as the minimum sample size [31] based on 36% prevalence reported by Akwuobu *et al*. [32], a total of 640 fecal samples were used in this study for buoyancy and accuracy of data. In the 60 randomly selected farms, 30 farms each were surveyed during the dry

and wet seasons. Stool samples were aseptically collected directly from the cloacae in 5-25% of chicken reared, using sterile swab sticks. Epidemiological variables such as farm location, season, type of poultry, sex, and flock size were determined and recorded. Thereafter, the fecal samples were transported in ice-packed container, within 1 h of collection, to the diagnostic laboratory of Veterinary Microbiology Department, University of Nigeria, Nsukka, for the bacteria isolation.

Isolation of thermophilic *Campylobacter* species

The fecal samples were plated directly on modified charcoal cefoperazone deoxycholate agar (mCCDA, CMO739, Oxoid, UK) for the *Campylobacter* isolation. The agar (mCCDA) was prepared according to the manufacturer's instruction and supplemented with mCCDA selective supplement (SRO 155E, Oxoid, UK) before plating. The setup was then incubated under microaerophilic conditions, generated by CampyGen® (CN 0025A, Oxoid, UK) at 42°C for 48 h, as already described [26]. Suspected thermophilic *Campylobacter* colonies appearing flat, glossy, and thinly spreading on the agar surface were sub-cultured on supplemented agar as described by Nwankwo *et al*. [12] to obtain a pure culture. Subsequently, putative pure colonies were subjected to biochemical characterization as described by Barrett *et al*. [33]. Colonies that tested positive during biochemical characterization were also Gram-stained and viewed for Gram-negative short rods, in oil immersion at 100× objective for confirmation.

Statistical analysis

Completed copies of the questionnaires were retrieved and the responses collated for statistical analysis. Fisher's exact test was performed to check for significant association ($p<0.05$) between TCI in poultry and farm location, type of poultry, sex, season, and flock size. The statistic was also used to determine whether there is a significant association ($p<0.05$) between *Campylobacter* infection in poultry and farm practices and epidemiological variables that could enhance dissemination of the infection and hence constitute the risk factors or determinants of TCI. Casual association between the infection and farm practices or epidemiological variables was established at $p<0.05$ and/or odds ratio values >1. The tests were performed at 5% probability level using GraphPad Prism® version 6.04 (GraphPad® Inc., San Diego, California, USA).

Results

Demographics and socioeconomic characteristics of respondents

Majority of the farm owners or managers were males aged <45 years (76.7%, 46/60). In addition to poultry keeping, most of the farmers reared other food animals, such as pig (38.4%, 23/60), small ruminants (35%, 21/60), fish (20%, 12/60), and cattle (7.7%, 4/60); while 15.4% kept dog or cat as biological control for rodents or security in the farm. About 68% (41/60) of the farmers had a flock size of <100 birds. About 5% (3/60) of the farmers had no formal education; while 41.7% (25/60), 40% (24/60), and 13.3% (8/60) of the respondents attained basic (primary), post-primary (secondary), and tertiary educational levels, respectively.

Management and biosecurity practices adopted in poultry farms surveyed

Table-1 contains the result of husbandry systems and biosecurity measures implemented in poultry farms surveyed. Majority of the farms (93.3%, 56/60)

Table-1: Husbandry and biosecurity practices adopted in poultry farms (n=60) surveyed in Nsukka agricultural zone, Nigeria.

Variables	Characteristics	Number of farms (%)
Husbandry systems	Intensive	56 (93.3)
	Semi-intensive	4 (5.0)
	Traditional (extensive)	1 (1.7)
Type of intensive system	Deep litter	49 (87.6)
	Battery cage	7 (12.5)
Biosecurity measures practiced	Availability of perimeter fencing	21 (35.0)
	Control of intrusion of wild or migratory birds into the farm	18 (30.0)
	Sanitization of drinking water	19 (31.7)
	Screening of poultry houses with net	29 (48.3)
	Practiced all-in-all-out stock replacement program	34 (56.7)
	Quarantine of incoming or exposed animals	18 (30.0)
	Enforcement of unidirectional movement in the farm	14 (23.3)
	Farm sited about 200 m away from residential areas and water bodies	13 (21.7)
	Practiced occasional fumigation	6 (10.0)
	Availability of functional rodents control programs	10 (16.7)
	Availability of hand washing facilities at farm entrances	8 (13.3)
	Use of only new or disinfected egg crates in the farm*	2 (3.9)
	Availability of functional foot dips at farm and pen entrances	11 (18.3)
	Routine vaccination against endemic important poultry diseases	53 (88.3)

*Not applicable to nine farms that did not rear laying birds

practiced intensive management system of poultry production, either as deep litter (87.6%, 49/56) or battery case system (12.5%, 7/60). Several biosecurity practices and various levels of compliance were observed (Table-1). Prominent among the practices is routine vaccination against endemic poultry diseases, practiced in 88.3% (53/60) of the farms surveyed.

Prevalence of thermophilic *Campylobacter*

Farm and individual prevalence of 78.3% (47/60) and 19.4% (124/640), respectively, were recorded for TCI. The prevalence of 15.7% (54/343) and 23.6% (70/297) were found for birds reared in urban and rural areas, respectively (Table-2). Similarly, the prevalence rates of 17.2% (79/460), 25% (45/180), 14.7% (49/334), and 24.5% (75/306) were documented for broiler, layer, male and female birds, respectively (Table-2). Thermophilic *Campylobacter* species were isolated from 23.9% (68/285) and 15.8% (56/355) of the fecal samples during the wet and dry seasons, respectively. Significant association ($p<0.05$) existed between TCI and farm location, type of poultry, sex, and season; but none was found at $p=0.548$ between the bacterial infection and flock size (Table-2).

Risk factors for thermophilic *Campylobacter* infection

Table-3 contains the results on risk factors for TCI in poultry farms surveyed. Major risk factors found and the proportions of farms involved were non-sanitization of drinking water 68.3% (41/60), rearing other animals alongside poultry 85% (51/60), non-quarantine of exposed or incoming birds 70% (42/60), keeping birds of different ages together 61% (37/60), thinning (partial depopulation of flock) 53.5% (32/60), overstocking 43.3% (26/60), and presence of rodents and/or wild

Table-2: Prevalence of thermophilic *Campylobacter* infection in poultry farms (n=60) surveyed in Nsukka agricultural zone, Nigeria.

Epidemiological variables	Number of sampled	Number of positive	Prevalence	Odds ratio	95% confidence interval	p-value
Farm locations						
Urban areas	343	54	15.7	0.6	0.41-0.9	0.0159*
Rural areas	297	70	23.6			
Types of poultry						
Broiler	460	79	17.2	0.622	0.41-0.94	0.0265*
Layer	180	45	25			
Sex						
Male	334	49	14.7	0.53	0.36-0.79	0.0019*
Female	306	75	24.5			
Season						
Wet	285	68	23.9	1.67	1.13-2.48	0.0118*
Dry	355	56	15.8			
Flock size						
<100 birds	298	61	20.5	1.14	0.77-1.69	0.5480
≥100 birds	342	63	18.4			

*Denotes statistically significant p-value, Fishers' exact test

Table-3: Risk factors for thermophilic *Campylobacter* infection in poultry farms (n=60) survey in Nsukka agricultural zone, Nigeria.

Risk practices	Number of farms involved	Number of farms infected	Proportion (%)	Odds ratio	95% CI	p-value
Non-sanitization of drinking water						
Yes	41	36	68.3 (41/60)	5.24	1.42-19.3	0.0164*
No	19	11				
Rearing of other animals alongside poultry						
Yes	51	42	85 (51/60)	3.73	0.83-16.7	0.0917
No	9	5				
Non-quarantine of exposed or incoming birds						
Yes	42	35	70 (42/60)	2.5	0.7-8.93	0.1811
No	18	12				
Rearing of birds of different ages						
Yes	37	31	61 (37/60)	2.26	0.65-7.86	0.2148
No	23	16				
Presence of rodents and or wild birds in the farm						
Yes	24	20	40 (24/60)	1.67	0.45-6.19	0.5336
No	36	27				
Thinning (partial depopulation of the flock)						
Yes	32	29	53.5 (32/60)	5.37	1.30-22.2	0.0256*
No	28	18				
Overstocking/overcrowding						
Yes	26	24	43.3 (26/60)	5.74	1.14-28.8	0.0280*

*Denotes statistically significant p-value, Fishers' exact test

birds in the farm 40% (24/60). Significant association ($p<0.05$) was noted between the bacteria colonization of poultry and non-sanitization of drinking water, thinning, and overstocking.

Discussion

Mostly (76.7%) young people were involved in poultry rearing, probably due to the high level of acceptance of poultry products across diverse ethnic backgrounds and religious beliefs. In addition, poultry production provides the highest turnover rate and the quickest returns on investment in the livestock subsector [34]. Hence, poultry farming has become a major animal protein-deficient gap filler and a very important aspect of the national economy, contributing substantially to job creation, poverty alleviation, and crime reduction, despite the attendant public health risks thereof.

The 19.4% overall prevalence found is significant, given that *Campylobacter* infection is a major threat to public health [34,35] and human health is intricately connected to that of animals. Concerted effort should be made to limit human exposure to the infection through food chain or contact with an infected animal. Good farm management practices such as intensive husbandry system and strict biosecurity are pivotal to curtail dissemination of pathogens of public health importance at the farm level. Since poultry is a potent source of human *Campylobacter* infection [8,11,35,36], proper treatment of poultry feces before disposal or use as feed (in piggeries or fisheries) or manure (in vegetable and fruit gardens) may be an effective control measure against the spread of these enteric zoonotic pathogens. As wild bird feces can contaminate the environment [37], adequate environmental sanitation and hygiene, especially in public places, neighborhoods, and recreational centers, should be prioritized, to restrict the proliferation of the agents in the environment and the possible onward transmission to humans.

The 19.4% prevalence is lower than 36% prevalence previously reported in the same study area about a decade ago [32]. This shows that the infection is on the decline. The decline may be attributed to the husbandry management system as most farms (93.3%) have now adopted intensive husbandry system of poultry production in the study area. Intensive husbandry management system has the advantage of low pathogen infectivity as against the extensive or free-range (organic) system that predisposes to diseases, especially in the tropics. Under intensive management system, the animals are shielded from climatic and certain epidemiological factors that facilitate acquisition and spread of infection in animal farms [38].

In addition, prophylactic administration of antibiotics, widely practiced in poultry farms nationwide [22], may have contributed in reduction of *Campylobacter* colonization of the animals in the farms surveyed. Given that, TCI does not usually result in clinical manifestation of disease in animals, and hence, antibiotics treatment may not be warranted; administration of antimicrobial agents for therapeutic or chemoprophylactic treatment of other invasive infections may help depopulate non-targeted organisms such as *Campylobacter* species. The resultant effect, therefore, is a reduction in colonization of poultry with TCOs, but the risks of the zoonotic transmission and development of antibiotics resistant strains of *Campylobacter* species still subsist [21].

The overall prevalence is also lower than the previous findings [7,12,25], in which the prevalence rates of 38.8%, 81.9%, and 30% were reported in other parts of the country. Furthermore, the prevalence of 23.9% and 15.8% found during the wet and dry seasons, respectively, in the study area differs from 30%, 31%, and 25% which were reported during wet, cold dry, and hot seasons in Sokoto, Northwest Nigeria [21]. The differences in the findings could be attributed to discrepancies in climatic or epidemiological factors at the various study locations, including husbandry systems, season, and health status of the animals. In addition, disparities in the researcher's experiences and capabilities in the bacterial isolation and characterization processes can be determining factors. The total number of samples investigated can influence the accuracy of research outcomes.

Higher prevalence of *Campylobacter* infection in layers (25%) than broilers (17.2%) may be attributed to coprophagia and pecking of vents which are common in layers. These attributes predispose to TCI or other infections transmitted through the feco-oral route. In addition, layers are usually kept for a longer period in the farm than other birds. The extended period of stay in the farm exposes them to *Campylobacter* infection much more than the broilers usually reared for 6-9 weeks. Furthermore, stress associated with egg production and hatching, which laying birds, usually undergo, tends to lower their immunity and predisposes them to infectious agents like TCOs.

Although it has been adjudged low, 19.4% overall prevalence is high and very significant from public health and food safety points of view. Thermophilic *Campylobacter* colonization of one in every five poultry reared in the study area portents great public health risk; as poultry and poultry products are cherished and widely accepted across the board in the study area. The use of pets (dog and cat) for biological control of rodents or for security purposes in the farms may facilitate *Campylobacter* infection of the pets for onward transmission to humans. The significance of the finding is that the contamination of poultry meat during processing may be high, and humans may easily acquire the infection through the consumption of undercooked poultry products or contact with infected birds at the poultry-human interface.

In addition, the significance of *Campylobacter* colonization of poultry entails not only contamination

of poultry products during processing but also drinking water sources through contamination of surface and subsurface water during disposal of slaughterhouse waste, effluents, and meat offal. *Campylobacter* organisms have been reported to survive in animal dung for over 10 months [39]. Furthermore, such environment contaminated with fecal matters from live chicken has been associated with the development of childhood diarrhea, especially in developing countries, where *Campylobacter* is hyperendemic [7,40].

Conclusion

High prevalence of *Campylobacter* infection in poultry, relished by all and sundry in the study area, portends great public health risks; as the infection can easily be transmitted to humans at the poultry-human interface and through the food chain. In view of the need to achieve optimum health for humans, animals, and the environment; strict biosecurity in poultry farms surveyed is imperative to curb TCI in poultry and hence the risk of human infection and the resultant public health and economic consequences.

Authors' Contributions

EON conceived and designed the study. ION and JCU collected and analyzed the samples. EON designed the questionnaire and carried out the statistical analysis. ION drafted the manuscript and EON revised it. All authors read and approved the manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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