

Full Length Research Paper

Epidemiology of *Campylobacter* species in poultry and humans in the four agricultural zones of Sokoto State, Nigeria

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A study was conducted to establish the epidemiology of *Campylobacter* species in the four agricultural zones of Sokoto. A total of 798 (506 cloacal and 292 fecal) swabs from poultry and humans respectively were screened and analyzed using standard culture isolation technique and biochemical characterization. A total of 152 (30%) and 160 (55%) were positive for *Campylobacter* spp. in poultry and humans respectively. The prevalence rates of 53, 28, and 18% were for *Campylobacter coli*, *Campylobacter lari* and *Campylobacter jejuni* in poultry while 39, 37 and 24% were for *C. coli*, *C. lari* and *C. jejuni* in humans, respectively. The prevalence rate of 30% was recorded in both chicken and guinea fowl, while 14, 56 and 50% were found in pigeon, ducks and turkey, respectively. The prevalence rates were slightly higher in males than females in both poultry and humans. There was no significant statistical association ($P>0.05$) between prevalence rate and species. The prevalence in agricultural zones revealed 42, 39, 28 and 13% in Gwadabawa, Isah, Sokoto and Tambuwal, respectively in poultry, while in humans, 65, 25, 50 and 70% were recorded in the same order. There was no significant statistical association ($P>0.05$) between prevalence rate and sex, but the association between prevalence and zones were statistically significant ($P<0.05$) in both poultry and humans. Poultry in the state have been shown to harbor *Campylobacter* spp. and may serve as reservoir of infection for humans. Humans independent of age and sex, were infected with *Campylobacter* spp.. The transportation of poultry together with humans in the same truck while moving birds from different locations to live bird markets should be discouraged. Adequate environmental sanitation and strict hygiene measures should be implemented in the poultry slaughter slabs and processing units in the state.

Key words: *Campylobacter* species, poultry, humans, agricultural zones, Sokoto State, Nigeria.

INTRODUCTION

Campylobacter species (formerly *Vibrio fetus*) were first associated with diseases of cattle and sheep at the beginning of 20th century. They are small curved or spiral-shaped gram negative bacilli that exhibit rapid

darting and spinning motion (WHO, 2002). They are neglected zoonotic disease agents with an increased frequency of isolation from man, food, water, animals and their products (Salihu et al., 2010; Ugboma et al., 2013).

The economic loss due to *Campylobacter* infection poses a challenge to food and livestock industries as they usually colonize the gastrointestinal tract of birds causing diarrhea, less feed conversion ratio, decrease egg production and mortality in day old chicks (Butzler, 2004; Ruiz-Palacios et al., 1981). The rate of infection in poultry is affected by seasons and the type of production system (Kapperud et al., 1993; Wallace et al., 1997). Thermophilic *Campylobacter* spp., mainly *Campylobacter jejuni* and to a lesser extent *Campylobacter coli* have been recognized as the most common bacteriological causes of gastroenteritis in animals and humans worldwide (Jones et al., 1931). The gastroenteritis caused by these species is associated with abdominal pain and discomfort which sometimes persist even after the diarrhea has stopped (Jones et al., 1931). Humans acquire infection through handling and consumption of undercooked poultry meat. However, in the developing countries more cases of infection in children have been associated with poor hygiene (Coker et al., 2002). Other risk factors include the consumption of food and water contaminated with untreated animal or human waste in addition to close proximity and contacts with farm animals (Coker and Adefeso, 1994; Cools et al., 2003; Lindmark et al., 2009). Cost implication of treatment, in addition to increased resistance of these *Campylobacter* spp. to antimicrobial agents has been a concern in public health and disease control (Hein et al., 2003; Tollefson et al., 1999).

The aim of this study was to establish the prevalence of *Campylobacter* species in poultry and humans in the four agricultural zones of Sokoto State, Nigeria.

MATERIALS AND METHODS

Ethical approval

The research was approved by the Ethical Committee of the Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto. Ethical clearance was obtained from the Ministry of Health, Usman Farouk Secretariat Sokoto, Sokoto State.

The study area

The study was carried out in Sokoto State, which is located in the extreme Northwestern Nigeria and lies between the latitudes 12°N to 58°N and longitudes 4.8°E to 6.54°E with annual average temperature of 28.3°C. The 23 Local Government Areas have been grouped into 4 agricultural zones (MANR Sokoto, 2000). The state shares boundaries with Zamfara State to the East, Republic of Niger to the North and Kebbi State to the West.

Sample size determination

The minimum sample size for this study was determined by the

formula $N = Z^2 p(1-p)/d^2$ (Thrusfield, 2005), where N=Sample size; Z=the score for a given interval which is 1.96 (S.E) at 95% confidence interval; P= known or estimated prevalence; d=5% level of precision. The prevalence rate of 38.8% in birds in Sokoto was used for poultry (Salihu et al., 2009) while 20% was estimated for humans. With the known prevalence, the minimum calculated sample size (n) required for the study in birds was $1.96^2 \times 0.39 \times 0.61/0.05^2=365$, while the minimum sample size required for humans was $1.96^2 \times 0.20 \times 0.80/0.05^2=245$.

Sampling in poultry

A minimum of one Local Government Area (LGA) was randomly selected from each of the four Agricultural zones of the state. Visits were made to live bird markets in each of the selected LGAs to seek approval and cooperation from the authorities of the market union and estimate the number of birds that were presented for sales and slaughter during the market days. For each of the live bird market, visits were made once in every 2 weeks to avoid repeat sampling as birds presented for sales are usually transported from one live bird market to another and at least 40% (2 in every 5) of birds counted were sampled at each visit. In zone that has slaughter slab/processing points, cloacal swabs were collected outside the two weeks that samples were routinely collected from the market to avoid sampling same birds twice at both sales and slaughter unit.

Sampling in humans

Visits were made to the randomly selected hospitals in the Local Government Areas (LGAs) selected from each agricultural zone. Introductory letters from the hospital service management board were presented to the Chief Medical Officers of the selected hospitals. Convenient sampling technique was used in the collection of faecal swabs after the assigned health workers have explained the purpose of the study to the patients and such consented.

Sample transportation and processing

The samples were placed in Amies transport media (CMO425, Oxoid), kept cold with the use of ice pack (Butzler, 2004) and transported to the Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto for analyses. Samples were plated directly onto modified Charcoal Cefaperazone Deoxycholate Agar (mCCDA) and incubated at 42°C for 48 h under microaerophilic condition generated by Campygen® (Oxoid, BR0056) in the anaerobic jar (Butzler, 2004).

Identification of *Campylobacter* spp.

The plates were examined for typical *Campylobacter* colonies, characterized by creamy or white, greyish, moist, flat or slightly raised extending along the streak line, or regular circular discrete colony (Atabay and Corry, 1998). All the distinct pure colonies were gram-stained and isolates were identified to species level using the standard *Campylobacter* spp. phenotypic identification tests (Atabay and Corry, 1998; Barrett et al., 1988; Quinn et al., 1994).

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Table 1. Prevalence of *Campylobacter* species in Humans and Poultry in Sokoto State.

Sample source	Total sampled	Total + (%)	<i>C. jejuni</i> (%)	<i>C. coli</i> (%)	<i>C. lari</i> (%)
Humans	292	160 (55)	38 (24)	63 (39)	59 (37)
Poultry	506	152 (30)	29 (19)	79 (52)	44 (29)
Total	798	312 (39)	67 (21)	142 (46)	103 (33)

Table 2. Prevalence of *Campylobacter* species in different Species of Poultry in Sokoto State.

Species	Total sampled	Total positive (%)	<i>C. Jejuni</i> (%)	<i>C. coli</i> (%)	<i>C. lari</i> (%)	χ^2 value	P value
Chicken	400	119 (30)	23 (19)	62 (50)	34 (29)	8.106	0.0878 (P>0.05)
G/fowl	67	20 (30)	3 (15)	10 (50)	7 (35)		
Pigeon	21	3 (14)	2 (67)	0	1 (33)		
Duck	16	9 (56)	0	7 (78)	2 (22)		
Turkey	2	1 (50)	1 (100)	0	0		
Total	506	152 (30)	29 (19)	79 (52)	44 (29)		

G/fowl: Guinea fowl.

Table 3. Prevalence of *Campylobacter* species in male and female birds in Sokoto State.

Sex	Total sampled	Total+ (%)	<i>C. jejuni</i> (%)	<i>C. coli</i> (%)	<i>C. lari</i> (%)	χ^2 value	P value
Male	257	84 (32)	17 (20)	44 (52)	23 (27)	2.315	0.1281
Female	249	66 (27)	11 (17)	35 (53)	20 (30)		P>0.05
Total	506	150 (30)	28 (19)	79 (52)	43 (29)		-

Statistical analysis

The results obtained were presented in tables and percentages. Chi-square (χ^2 -test) was used to determine any significant statistical association between *Campylobacter* prevalence in poultry and humans with some categorical variables such as species, sex, and agricultural zone.

RESULTS

Out of the 798 samples analyzed, 506 were from poultry and 292 from humans. A total of 312 samples were positive for *Campylobacter* spp.; 152 (30%) and 160 (55%) in poultry and humans, respectively. The prevalence rates of 52, 29 and 19% were for *Campylobacter coli*, *Campylobacter lari* and *C. jejuni*, respectively in poultry while 39, 37 and 24% were for *C. coli*, *C. lari* and *C. jejuni*, respectively in humans (Table 1). The prevalence rate of 30% was recorded in both chicken and guinea fowl while 14, 56 and 50% were for pigeon, ducks and turkey, respectively (Table 2). In chicken, *C. coli* had the prevalence rate of 50% which is higher than 29 and 19% recorded for *C. lari* and *C. jejuni*, respectively. *C. coli* also recorded high rates in guinea fowl and ducks with 50 and 78%, respectively (Table 2).

In poultry, 84 (32%) and 66 (27%) prevalence rates were recorded for males and females, respectively while in humans, 70 (56%) and 89 (55%) were positive for males and females, respectively (Tables 3 and 4). *C. coli* had a higher prevalence rates than other species of *Campylobacter* in both male and female poultry. In humans, the same prevalence rates were recorded for *C. coli* and *C. lari* in males while *C. coli* had higher rate than others in female (Table 4). The zonal prevalence revealed 42, 39, 28 and 13% in Gwadabawa, Isah, Sokoto and Tambuwal zones, respectively in poultry while in humans, 65, 25, 50 and 70% were recorded in the same order (Tables 5 and 6). There was no significant association (P>0.05) between prevalence rate, species and sex in both poultry and humans, but the association (P<0.05) between prevalence and zone were statistically significant.

DISCUSSION

The prevalence of *Campylobacter* spp. in both poultry and humans has been established in the study area. The 30% prevalence rate in poultry was lower than 38.8% recorded in indigenous chicken in Sokoto by Salihu et al. (2009). The reduced rate could be an indication of

Table 4. Prevalence of *Campylobacter* species in males and females humans in Sokoto State.

Sex	Total sampled	Total+ (%)	<i>C. jejuni</i> (%)	<i>C. coli</i> (%)	<i>C. lari</i> (%)	χ^2	P value
Male	126	70 (56)	14 (20)	28 (40)	28 (40)	0.00218	0.9628
Female	161	89 (55)	24 (27)	34 (38)	31 (35)		P>0.05
Total	287	154 (54)	38 (25)	62 (40)	59 (38)		

Sex was not indicated in 5 faecal samples

Table 5. Prevalence of *Campylobacter* species in poultry in the selected Zones/Local Government Areas of Sokoto State.

Zone	LGA	Total sampled	Total+ (%)	<i>C. jejuni</i> (%)	<i>C. coli</i> (%)	<i>C. lari</i> (%)	χ^2 value
Sokoto	S. North	156	42 (27)	7 (17)	19 (45)	16 (36)	19.795
	Dange Shuni	116	33 (28)	14 (42)	11 (33)	8 (24)	
Gwadabawa	Illella	63	20 (32)	3 (15)	13 (65)	4 (20)	P=0.0014
	Silame	43	22 (51)	0	18 (82)	4 (20)	
Tambuwal	Yabo	53	7 (13)	0	3 (43)	4 (57)	P < 0.05
	Tambuwal	-	-	-	-	-	
Isah	Wurno	75	29 (39)	5 (17)	17 (58)	7 (24)	
	Rabah	-	-	-	-	-	
Total		506	153	30	82	43	

Table 6. Prevalence of *Campylobacter* species in humans in the selected Zones/Local Government Areas of Sokoto State.

Zone	LGA	Total sampled	Total+ (%)	<i>C. jejuni</i> (%)	<i>C. coli</i> (%)	<i>C. lari</i> (%)	χ^2 value
Sokoto	S. North	-	-	-	-	-	18.321
	Denge Shuni	158	79 (50)	35 (44)	23 (29)	21 (26)	
Gwadabawa	Illella	49	32(65)	3(9)	13 (41)	16 (50)	P=0.0004 P <0.05
	Silame	-	-	-	-	-	
Tambuwal	Yabo	-	-	-	-	-	
	Tambuwal	61	43 (70)	0	25 (58)	18 (42)	
Isah	Wurno	-	-	-	-	-	
	Rabbah	24	6 (25)	0	1 (17)	5 (83)	
Total		292	160	38	66	60	

increased awareness and improved environmental sanitation at backyard poultry houses at different homes, live bird markets and poultry farms. The prevalence rate in poultry was also in agreement with that of Uaboi-Egbenni et al. (2008) that recorded 33% prevalence rate in Lagos. However, it differs with the high prevalence rates of 94.2 and 89% recorded by Workman et al. (2005) and Georgios et al. (2004) in chicken meat and faeces, respectively. The similarities and variations in the prevalence rates could be a reflection of environmental

contamination, however, other factors such as stock density, season, feeding regimen and geographical location have been proposed to account for significant differences and similarities in the isolation rates (Mary et al., 2004; Stern, 1994).

The rate of prevalence among species of poultry was high in ducks, which is a water fowl. Ducks are known to tip up on the surface of shallow water or submerge completely and swim under the water in search of food. They get infected especially when the ground water is

contaminated with *Campylobacter* spp. (Savill et al., 2001). The low prevalence rate recorded in chicken might be linked to the free range system which is common in the study area as coprophagy which enhances bird to bird spread is limited. This can be supported by findings of Robino et al. (2010) with a *Campylobacter* spp. prevalence rate of 78.4% in intensively reared poultry and 18.3% in small scale rural poultry farming in Italy. The prevalence rates in pigeon, turkey and guinea fowl also revealed the possibilities of infection through feeds as they usually feed on insects, fruits, seeds and flowers which have been suggested as potential routes of infection in poultry (Waldenstrom et al., 2002). The interaction of these birds among themselves and with human communities suggests the possibilities of infection and transmission to humans (Shane, 1992; Waldenstrom et al., 2002). The higher prevalence of *C. coli* than other species in poultry in this study agreed with the findings of Wiczorek et al. (2012) that revealed 58.9% as *C. coli* and 41.1% as *C. jejuni*. Other reports on the higher isolation rate of *C. coli* compared to *C. jejuni* have also been reported (Kurincic et al., 2005; Lynch et al., 2011). However, the findings disagreed with the higher isolation rate of *C. jejuni* than other species in the work of Salihu et al. (2009) that reported 72.9% of the total isolate from chicken as *C. jejuni* and Cuiwei et al. (2001) who recorded the prevalence rate of 53.6, 41.3 and 5.1% for *C. jejuni*, *C. coli* and other species, respectively. Such differences have been attributed to several factors, including isolation method, sample size, seasonal variation and geographical location (Allos, 2001; Stanley et al., 1998). Since contact and consumption of contaminated improperly cooked poultry meat has been attributed to the occurrence of gastroenteritis in humans, the prevalence rate in poultry may have contributed to the prevalence in humans as over 80% of human population in the state are engaged in agriculture (Corry and Atabay, 2001).

The prevalence rate of 55% in humans disagreed with 78.4% recorded in Salihu (2009) study in the same study area and that of 87% among livestock workers as recorded by Saenz et al. (2000) in Spain. Salihu et al. (2009) collected and analyzed samples from a risk group (Poultry processors) while the focus of this study was on diverse groups which included people attending outpatient and ante natal clinics in the hospitals. *C. coli* had higher rate of 39% which contradicts the findings of workers who observed *C. jejuni* as the most common species of *Campylobacter* in humans (Ohanu and Offune, 2009; Salihu, 2009). *C. coli* also had high rate of 51% in chicken which was in agreement with the record of 58.9% as *C. coli* and 41.1% as *C. jejuni* by Wiczorek et al. (2012). It has been observed that *C. lari* is mostly found in wild birds and its isolation has remained low both in humans and poultry (Benjamin et al., 1983). However, the isolation rate for *C. lari* in poultry in this study was in agreement with that of 28% by Baserisalehi et al. (2007)

in Iran. Furthermore, the lower isolation rate of *C. lari* to *C. coli* in this study was in agreement to the work of Uaboi-Egbenni et al. (2008) who reported a zero rate of *C. lari* and 14.2% for *C. coli*. The prevalence of *Campylobacter* spp. may be dependent on the sample size and weather conditions of different areas as some species grow optimally during the hot temperature and high humidity. Other species such as *Campylobacter hyointestinalis*, *Campylobacter sputorum* and *Campylobacter fetus* not found in the study were likely due to the high temperature of birds that do not support their survival, agents in the selective medium such as cefoperazone that might have hindered their growth and unsuitable temperature at 42°C used in the isolation (Martin et al., 2002).

The prevalence rates as recorded in the agricultural zones can be used as a reflection of environmental contamination in the areas. The high prevalence rates in poultry and humans recorded in Gwadabawa zone can be linked to the presence of large live bird market at Niger/Nigeria border town of Illella located in this zone whereby poultry that were transported unchecked into the country may have served as carriers of infection for humans.

High and low prevalence rates for humans and poultry, respectively in Tambuwal zone suggest the possibilities of other source of infection in humans other than poultry (Cools et al., 2003; Ugboma et al., 2013). However, genetics studies are needed to further link the isolates from poultry and humans. There was no much difference in prevalence rates in male and female birds and humans which is in agreement with the findings of Samuel et al. (2004) that recorded similar rates suggesting no sex preference in *Campylobacter* infection.

Conclusion

The study has established the prevalence of *Campylobacter* spp. in both poultry and humans in Sokoto State. Poultry and humans were infected independent of species and sex while different prevalence rates were recorded in different agricultural zones. Hence, human activities like the transportation of poultry together with humans in the same truck while moving birds from different Local Government Areas to live bird markets should be discouraged. Adequate environmental sanitation and strict hygiene measures such as washing of hands after handling of live birds, raw poultry meat especially for those that work in poultry slaughter slabs should be implemented to avoid the spread of *Campylobacter* infection in the state.

Conflict of interests

The authors have not declared any conflict of interests.

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