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Epidemiology of Thermophilic *Campylobacter* Species in Rural Poultry in Kebbi State, Nigeria

Abubakar SM Abba Maiha[1]*, YA Adamu[2], AO Talabi[3] and MB Abubakar[4]

[1]*Department of Animal Health and Production Technology, Federal Polytechnic Mubi. Adamawa State. Nigeria*

[2]*Department of Veterinary Medicine, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto, Nigeria*

[3]*Department of Veterinary Medicine and Surgery, Faculty of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria*

[4]*Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto, Sokoto State, Nigeria*

***Corresponding Author:** Abubakar SM Abba Maiha, Department of Animal Health and Production Technology, Federal Polytechnic Mubi. Adamawa State. Nigeria.

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Abstract

A study was conducted to determine the prevalence of thermophilic Campylobacter species in the four Emirate councils of Kebbi State. A total of 400 cloacal swabs from domestic birds were screened and analyzed using standard culture isolation technique and biochemical characterization. A total of 177 (44%) were positive for Campylobacter species. The prevalence of 89%, 51% and 37% were recorded for *Campylobacter coli, C. lari* and *C. jejuni* respectively.

The prevalence of 46% and 38% were recorded in chicken and guinea fowl, while 28%, 53% and 50% were found in pigeon, duck and turkey respectively. Sex specific prevalence was slightly higher in males than females. Using Chi square analysis, there was no statistical significant association between sex, species and infection (p-value > 0.05). The prevalence in the four Emirate councils revealed 58%, 53%, 36% and 30% in Gwandu, Argungu, Yauri and Zuru respectively. There was statistical association between the selected local governments in the Emirate councils and infection (p-value < 0.05). Poultry in the state have been shown to harbor *Campylobacter* species and may serve as reservoir of infection for others animals and humans. The transportation of poultry along with human passengers in the same vehicle 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 backyard poultry houses, slaughter slabs and processing units in the state.

Keywords: *Epidemiology; Campylobacter; Poultry*

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Introduction

Campylobacter is a major cause of human enteritis, and food-borne Campylobacteriosis is considered a main problem of Public Health in many developed countries. Poultry products are suspected to be an important source of infection in many countries (Anon., 2000). The thermophilic or thermotolerant *Campylobacter* are those that require a slightly higher temperature for their growth than other Campylobacter species and they include; *C. jejuni, C. coli* and *C. lari* found in avians (Skirrow, 1994; Rosef., *et al*. 2001). Avian carriage of Campylobacter has been regarded as a potential hazard to animals and human health, either through consumption of raw or undercooked carcass or by contamination of water supplies (Varslot., *et al*. 1996). A wide variety of avian species, including domestic Chickens, turkeys, ducks, pigeons, quail, waterfowls, geese and ostriches, harbor *Campylobacter* species (Broman., *et al*. 2004).

However, they are unevenly distributed among species and the feeding behaviour of birds has been shown to influence the *Campylobacter* colonization rate (Alterkruse., *et al*. 1999; Waldenstrom., *et al*. 2003). The Nigerian domestic birds are raised on a small scale in most households in rural and semi-urban areas of Northwestern Nigeria. They are free range poultry (rural poultry) made up of mostly chickens, ducks and guinea fowls, which are domesticated for the purpose of eggs and meat production.

These birds are managed under the extensive systems (Emikpe., *et al*. 2005), and are usually not vaccinated (Adu., *et al*. 1986). It is widely believed that they act as reservoirs of most important poultry diseases, including *Campylobacter* infection (Bouzoubaa., et al. 1992). Chickens (*Gallus gallus domesticus*) are the most important of the free range poultry species in terms of number and development (Oluyemi and Roberts, 1979). They are kept by over 90% of rural households, especially women, as assets (Ajala., *et al*. 2007), providing an important source of high quality protein as well as a source of income for the families (Abubakar., *et al*. 2008).

Poultry being one of the sources of infection in animal and human, ecological approach is required to understand the epidemiology of the infection (Engberg., *et al*. 2001; Alfredson and Korolik, 2007; Han., *et al*. 2007). The aim of the study was to determine the prevalence of thermophilic *Campylobacter* species in rural poultry in Kebbi State, Northwestern Nigeria.

Materials and Methods

Study area

Kebbi State is geographically located to the North Western part of Nigeria at 11° 30'N 4° 00'E. Kebbi State falls within the Sudan Savanna with mean minimum temperature of 26°C (Kowal and Knabe, 1992). During the harmattan season (December to February), the temperature can go down to as low as 21°C and mean maximum temperature can go up to 40°C during the months of April to June (MANR, 1999). Annual rainfall is about 800mm and relative humidity is low (40%) for most of the year except during the wet season when it reaches an average of 80%. The wet season lasts from June to September, the hot season April to June while cool dry season lasts from December to February (Odjugo, 2010). Kebbi State was ranked among the five states with the highest number of livestock in Nigeria. Agriculture is the main occupation of the people especially in rural areas (animal rearing and fishing).

Research design

The study was a cross sectional study of *Campylobacter* infection in domestic birds. One hundred samples were collected from domestic birds at poultry markets from each of the selected four local government areas (Argungu, Birni Kebbi, Yauri and Zuru). Each was selected from one of the four local governments in the state. Random sampling techniques were used in sample collection.

Sampling method

Domestic birds at live bird Markets were the target population while poultry at live Bird Markets were the sampling frame. Purposive sampling as described by Paul (2006) was used for selection of local governments' areas while simple random sampling as described by Valerie and John (1997) was used for sampling domestic birds in selected areas.

Sample size determination

The minimum sample size for this study was determined by the formula,

$n = t^2 x \underline{p}^{exp}(\underline{1-p}^{exp})/\underline{d}^2$ (Thrusfield, 2005)

Where n = sample size, t^2 = the score for a giving interval which is 1.96 (S.E) at 95%, confidence interval, p^{exp} = Known or estimated prevalence, and d^2 = precision at 0.05.

The samples were calculated at 38.8% prevalence, (Salihu., *et a*l. 2009) at 95% confidence interval, with desired precision of 5%.

$n = (1.96)^2 \times 0.39 \times (1-0.39)/(0.05)^2$,

$n = 0.9139/0.0025 = 356.5$

$n = 366$

For more precision of the study, 400 samples were collected.

Thus, n = 400

Sample collection

Permission was obtained from the Ministry of Agriculture and Natural Resources and for each of the selected market 2 in every 5 bird (40%) counted were randomly sampled. A total of 400 domestic birds were sampled at poultry markets from four of the randomly selected local government, each from one of the four Emirate councils in the state. Cloacal swabs or freshly voided faeces were collected using sterile commercial swab sticks and were placed in Amies transport media, kept cold with the use of ice blocks (Butzler, 2004). Samples were transported within few hours after collection on the same day to the Veterinary Microbiology Laboratory, Faculty of Veterinary Medicine, Usmanu Danfodio University, and Sokoto for processing.

Processing of samples

Samples were inoculated directly onto a selective medium, modified charcoal cefaperazone Deoxycholate Agar (mCCDA) and incubated at 42°C for 48 hrs (Butzler and Megraud, 2002). Suspected Campylobacter colonies on the selective mCCDA medium were identified based on their characteristics features as creamy or white, moist, flat or slightly raised, extending along the streak line, or regular circular discrete colony based on the description of Atabay and Corry (1998).

Suspected *Campylobacter* isolates were confirmed based on their biochemical reactions as follows: Oxidase test, Hippurate hydrolysis test, Catalase test, Hydrogen sulphide production test (Atabay and Corry, 1998) and sensitivity to Cephalothin, Nalidixic acid using agar disc diffusion method (CLSI, 2014.

Results

Out of the 400 samples analyzed, a total of 177 samples were positive for *Campylobacter* spp. The prevalence of 89%, 51% and 37% were recorded for *C. coli, C. lari* and *C. jejuni*, respectively (Table 1). The prevalence of 46% and 38% was recorded in both chicken and guinea fowl while 28%, 53% and 50% were recorded for pigeon, ducks and turkey respectively (Table 1). *C. coli* recorded high rates in guinea fowl and ducks with 60 and 64%, respectively (Table 1).

Sex-specific prevalence of 98 (47%) and 79 (40%) were recorded for males and females, respectively (Table 2). *C. coli* had a higher prevalence than other species of *Campylobacter* in both male and female (Table 2). Species to specific prevalence in the selected local governments showed that *C. coli* had the prevalence of 89(22.3%) which is higher than 51(12.8%) and 37(9.3%) recorded for *C. lari* and *C. jejuni,* respectively (Table 3). The prevalence per *Emirate council* in Kebbi State has recorded 58%, 53%, 36% and 30% in Gwandu, Argungu, Yauri and Zuru Local governments respectively (Table 4). Figure 1 represent percentage prevalence of Campylobacter per local governments. There was no association ($P > 0.05$) between prevalence rate, species and sex in poultry, but the association ($P < 0.05$) between prevalence and local governments were statistically significant.

Species	Total sampled	Total positive (%)	*C. jejuni* (%)	*C. coli* (%)	*C.lari* (%)
Chicken	278	129 (46)	32 (24.8)	61 (47.3)	36 (27.9)
Guinea fowl	52	20 (38.5)	2 (10)	12 (60)	6 (30)
Pigeon	36	10 (27.8)	2 (20)	4 (40)	4 (40)
Duck	32	17 (53)	1 (5.9)	11 (64)	5 (29.4)
Turkey	2	1 (50)	0 (0)	1 (50)	0 (0)
Total	400	177 (44)	37 (9.3)	89 (22.3)	51 (12.8)

$\chi^2 = 6.237$ p = 0.182 p > 0.05

Table 1: *Prevalence of Campylobacter species in different species of domestic birds in Kebbi State.*

Sex	Total Number sampled	Total Number positive	Species		
			C. jejuni (%)	*C. coli* (%)	*C.lari* (%)
Male	207	98(47)	21(21.4)	51(52.0)	26(26.5)
Female	193	79(40.9)	16(20.0)	38(48.1)	25(31.6)
Total	400	177(44)	37	89	51

$\chi^2 = 1.664$ p = 0.1971 p > 0.05

Table 2: *Sex specific prevalence of Campylobacter infection in Kebbi state.*

LGA	Total Sampled	Total Positive (%)	*C. jejuni*	Species *C. Coli*	*C. lari*
Argungu	100	53	10 (18.9)	26 (49.1)	17 (17.1)
Birni Kebbi	100	58	13 (22.4)	29 (50)	16 (27.6)
Yauri	100	36	8 (22.2)	17 (47.2)	11 (30.6)
Zuru	100	30	6 (20)	17 (23.3)	7 (23.3)
	400	177	37 (9.3)	89 (22.3)	51 (12.8)

Table 3: *Species to Specific Prevalence of Campylobacter infection in domestic birds in the selected local government areas.*

Emirate Councils	LGA	Total Sampled	Total Positive (%)	Total Negative (%)
Argungu	Argungu	100	53	47
Gwandu	Birni Kebbi	100	58	42
Yauri	Yauri	100	36	64
Zuru	Zuru	100	30	70
	Total	400	177	223

$\chi^2 = 21.758$ p = 0.001 p < 0.05

Table 4: *Prevalence of Campylobacter infection in domestic birds in the selected local government areas.*

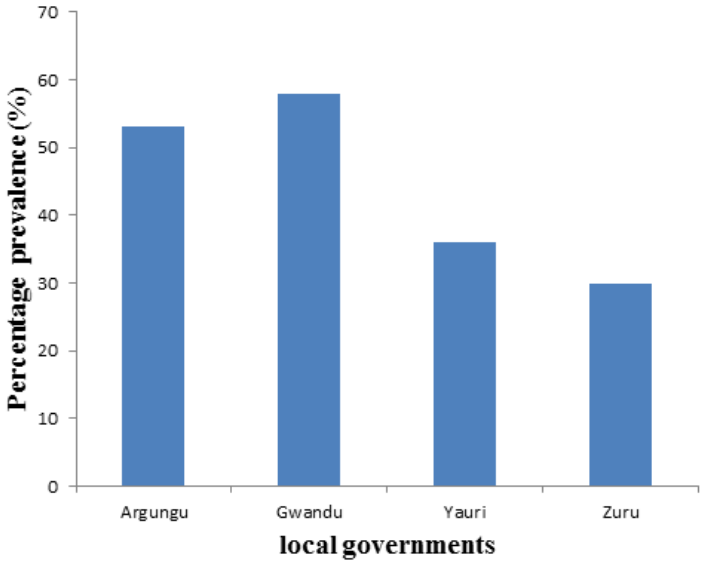

Figure 1: *Percentage prevalence of Campylobacter infection per local governments.*

Discussion

The prevalence of *Campylobacter* species in domestic birds has been established in the study area. The 44% prevalence in domestic birds was higher than 30% recorded in domestic birds by Nwankwo., *et al.* (2016) in Sokoto State and 33% recorded in poultry in Lagos State by Uaboi-Egbenni., *et al*. (2008). The increased rate could be due to lack of awareness and poor environmental sanitation at backyard poultry houses at different homes, live bird markets and poultry farms.

The prevalence rate in domestic birds was also in agreement with that of Salihu., *et al*. (2009) that recorded 38.8% prevalence in Sokoto State. 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).

The prevalence among species of domestic birds 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 species (Savill., *et al*. 2001). The low prevalence recorded in chickens 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* species prevalence of 78.4% in intensively reared poultry and 18.3% in small scale rural poultry farming in Italy. The prevalence in pigeons, turkeys and guinea fowls also revealed the possibilities of infection through feeds as they usually feed on insects, fruits, seeds and flowers which have been suggested as potential sources of infection in poultry (Waldenstrom., *et al*. 2003). The findings in this study showed no statistical association between *Campylobacter* infections in domestic birds at the study area to species of birds.

The higher prevalence of C. coli than other species in this study agreed with the findings of Nwankwo., *et al*. (2016) who reported the prevalence of 53%, 28% and 18% for *C. coli, C. lari* and *C. jejuni* in domestic poultry respectively and Wieczorek., *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 (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 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 (Stanley., *et al*. 1998; Allos, 2001). *Campylobacter coli* also had high rate of 50% while C. jejuni had the lowest rate in chicken which was in agreement with the record of 50%, 29% and 19% as *C. coli, C. Lari* and *C. jejuni* respectively by Nwankwo., *et al*. (2016) in Sokoto State.

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 i*n 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 species 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 *C. hyointestinalis, C. sputorum* and *C. 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 different prevalence rates as recorded in the Local governments can be used as a reflection of weather conditions and environmental contamination in the areas. High prevalence rates were recorded in Gwandu and Argungu while low prevalence rates were recorded in Yauri and Zuru Local governments respectively.

There was no statistical significant difference in prevalence rates in male and female birds which is in agreement with the findings of Nwankwo., *et al*. (2016) and Salihu., *et al*. (2009) that recorded similar rates suggesting no sex preference in Campylobacter infection.

Conclusion

The study revealed a total of 177 (44%) samples, were positive for Campylobacter species. The prevalence of 89%, 51% and 37% were recorded for *C. coli, C. lari* and *C. jejuni*, respectively. *Campylobacter co*li were more prevalence than *C. jejuni*. Domestic birds were infected independent of species and sex while different prevalence rates were recorded in different Local governments.

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