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Prevalence of *Campylobacter* species in fecal samples of pigs and humans from Zuru Kebbi State, Nigeria

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Abstract

Aim: The study was carried out to survey and determine the prevalence of *Campylobacter* species among pigs and humans within the pig rearing areas of Zuru Kebbi State, Nigeria.

Materials and Methods: A cross-sectional study was conducted among household pigs and humans between September, 2013 and February, 2014. Isolation and characterization of *Campylobacter* species were performed using standard culture isolation techniques and biochemical characterization. A total of 450 fecal samples comprised of 300 from pigs and 150 from humans was collected and analyzed.

Results: Prevalence of *Campylobacter* were 278 (92.67%) and 94 (62.67%) for pigs and humans respectively. The most encountered *Campylobacter* species in both cases was *Campylobacter coli* (276 [74.19]) followed by *Campylobacter jejuni* (62 [16.66]). The least isolated species in pigs was *Campylobacter hyointestinalis* 5 (1.8%) while *Campylobacter lari* 2 (2.13%) was least isolated in humans. *C. lari* was not found in pigs. No significant association ($p>0.05$) existed between *Campylobacter* isolates and the age and sex of both pigs and humans in this study.

Conclusion: Both pigs and humans within the pig rearing areas of Zuru have been shown to harbor *Campylobacter* species and this might be due to extensive system of pig farming with indiscriminate defecation by pigs coupled with unhygienic disposal of human wastes in the environment and poor personal hygiene.

Keywords: *Campylobacter* species, humans, pigs, Zuru.

Introduction

Campylobacter, *Gram-negative*, small curved spiral rods of 0.2-0.5 μm long with a single unsheathed polar flagellum [1], sometimes present in large numbers in the small intestine of most animals including pigs. They are the causative agents of campylobacteriosis in animal and human which are of public health importance, especially *Campylobacter* enteritis due to *Campylobacter jejuni* and *Campylobacter coli* [2]. Due to increasing incidence, expanding spectrum of infections, potential of HIV-related deaths due to *Campylobacter*, and the availability of a complete genome sequence of *C. jejuni* NCTC11168, interest in campylobacteriosis research and control in developing countries is growing [3]. Campylobacteriosis is known as the most common cause of severe form of bacterial diarrhea worldwide [4] and the most frequently reported cause of gastrointestinal infection in man exceeding cases of salmonellosis and shigellosis [5]. This infection occurs mainly in infants, elderly people and patients with underlying disease. The disease in humans is accompanied by fever, bloody diarrhea, headache and abdominal pain, also nausea and vomiting may occur. In immunocompromised individuals it may spread to the blood stream and become life-threatening [6].

It is widely assumed that campylobacteriosis is primarily a food-borne disease in which food of animal origin such as poultry and pork play a major role [2] and of which wild and domestic animals, particularly birds and pigs serve as major reservoirs [6]. It has been estimated that 500 cells of *C. jejuni* can cause human illness [2]. This means that even very small number of *Campylobacter* cells in water or food may be a potential health hazard. The classical clinical signs of campylobacteriosis in pigs include, sporadic abortion in sows and birth of weak or dead piglets [7, 8], mild and sometimes creamy diarrhoea in piglets that last for several days which often leads to death. The resultant effect of the observed signs often leads to a decrease in productivity and subsequent economic losses.

The unhygienic and poor nature of the farrowing pens in many developing countries [9,10], as observed in the study area, the semi-intensive system of pig rearing in the study area can facilitate the spread of

Campylobacter among pigs and from poultry to pigs, since poultry birds are allowed to roam freely in search of feeds. Eating of unhygienically or insufficiently cooked pork, carcass contamination during slaughter, and roaming-pets that bring infection home contributes greatly as a source of *Campylobacter* infection in humans [11]. Furthermore, there is close contact and interactions between pigs, humans and other animal species in the study area, coupled with the fact that there is no baseline information on the prevalence of *Campylobacter* species in the study area, then study of this kind becomes imperative.

Materials and Methods

Ethical approval

Ethical clearance as approved by the Research Ethical Committee of the Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto was obtained prior to this study.

Sampling sites

The study was carried out within the pig rearing areas of Zuru Kebbi State, Nigeria, between September, 2013 and February, 2014. Zuru lies in the southern part of Kebbi State on the latitude 11°5'-11°56'N and longitude 4°3'-5°25'E. The areas lie within Sudan savannah and has mean annual rainfall of 1000 mm [12]. The human population is estimated at 165,547 with land area of 653 km^2 [13]. People in the study area engage in crop farming, hunting, blacksmithing, livestock rearing such as cattle, sheep, goats, and pigs. Pigs are raised semi-intensively and serve as a source of income for the households [14].

Sample size determination

There is lack of data on the prevalence of *Campylobacter* species from the study area. Therefore, sample size for the pig population was calculated at 20% prevalence [15] and a prevalence of 5% as obtained by Coker *et al.* [3] in Lagos State, Nigeria was used for humans at 95% confidence interval, with a desired precision of 5% using

$$n = t^2 \times P^{exp} (1-P^{exp})/d^2 \text{ [16].}$$

n=Sample size, t=1.96 (S.E), P=Prevalence, d=Level of precision (5%).

A minimum of 245 and 72.9 sample sizes was derived for pigs and humans respectively, and were also respectively adjusted to 300 and 150 so as to increase detection chances. Thus, a total of 450 fecal samples was collected from both pigs and humans.

Sample collection and transportation

A convenient sampling technique was employed to collect fecal samples from pigs and humans. Fecal samples were collected using sterile swab sticks per rectum from restrained pigs. Fecal sample from volunteer pig farmers and their household members were also collected using sterile swab sticks within the first 4 h of defecation. Age was determined by dentition [17] coupled with information from pig owners and sex was determined by physical examination of external genitalia. Both age and sex were recorded at the point of collection, and fecal samples collected were transferred into bijou bottles containing Amies transport (pre-enrichment) media. The samples were transported in an ice-packed container within 3-4 h of collection to the Veterinary Public Health Laboratory, Usmanu Danfodiyo University, Sokoto, Nigeria.

Sample processing and isolation

The fecal sample was plated onto cefoperazone charcoal deoxycholate agar, using the swab stick with fecal matter and incubated at 42°C for 48 h under microaerophilic condition generated by a gas generating pack (Campygen CN25, Oxoid).

Phenotypic identification of *Campylobacter* spp.

The plates were examined for typical *Campylobacter* colonies, characterized by greyish, creamy/white, flat, moistened colonies with tendencies to spread. All the distinct colonies found were gram-stained, and different biochemical tests such as catalase, oxidase, hippurate hydrolysis and H_2S production in triple sugar iron agar tests were performed. The isolates were identified to species level using the standard *Campylobacter* phenotypic identification tests as recommended by Atabay and Corry [18]. *C. jejuni* among the confirmed isolates was identified using the hippurate hydrolysis test [19]. A small amount of pure culture was inoculated in 0.4 ml of 1% sodium hippurate (1 g of sodium hippurate and 99 ml of distilled water) in a tube. The tube was caped and incubated for 2 h at 37°C. Then, 0.2 ml of 2% ninhydrin solution was added and re-incubated for further 15 min at 37°C. The development of a purple-violet color identified the isolate as *C. jejuni.*

Antibiotic sensitivity test was determined using the disc diffusion method as described by Bauer *et al.* [20]. The antibiotic discs used were; nalixidic acid (30 mg), cephalothin (30 mg) and metronidazole (30 mg).

Statistical analysis

The results obtained were presented in tables and percentages. Chi-square (χ^2-test) was used to test for any significant association between *Campylobacter* isolates from pigs and humans feces with some epidemiological variables such as age and sex.

Results

Out of the 450 samples that were collected and analyzed, 372 (82.6%) were positive for *Campylobacter* organisms. 278 (92.66%) of the 300 pigs' feces tested, and 94 (62.66%) of the 150 humans' feces tested were positive. Of the identified *Campylobacter* species, *C. coli* has the highest isolation rate of 276 (74.19%), while *Campylobacter lari* has the least rate of 2 (0.54%). Other species isolated were *C. jejuni* 62 (16.66%), *Campylobacter*

upsaliensis 22 (5.91%), and *Campylobacter hyointestinalis* 10 (2.69%), while *C. lari* was not isolated from pig fecal sample (Table-1).

As shown in Table-2, the highest number of samples (160 [53.33%]) were collected from the male pigs with a total of 144 (90.0%) positive for *Campylobacter* species. However, female pigs have the higher percentage frequency (95.71%) than the males. With respect to age of the pigs sampled, adult pigs have a higher percentage (137 [94.48%]) of positive samples than the young pigs (Table-3). There was no significant association ($p>0.05$) between the isolates, age and sex of the pigs.

For humans, the highest number of samples (72 [48.0%]) were collected from people between the ages of 10 and 29 years with 44 (61.11%) positive samples, while the lowest number (34 [22.66%]) of samples collected were obtained from people who are 30 years and above, however with highest positive samples of 25 (73.53%) as shown in Table-4. In relation to the sex of the people, higher isolates, 46 (64.79%) were obtained from the female. Furthermore, there was no significant association between the isolates and ages and sex of the people (Table-5).

Table-1: Percentage isolation of *Campylobacter* species in pigs' and humans' fecal samples from Zuru Kebbi State.

***Campylobacter* (C) species**	**Percentage isolates from pigs (%)**	**Percentage isolates from humans (%)**	**Total isolates (%)**
C. coli	219 (78.71)	57 (60.63)	276 (74.19)
C. jejuni	39 (14.03)	23 (24.50)	62 (16.66)
C. upsaliensis	15 (5.40)	7 (7.45)	22 (5.91)
C. hyointestinalis	5 (1.80)	5 (5.32)	10 (2.69)
C. lari	0 (0.00)	2 (2.13)	2 (0.54)
Total	278 (92.66)	94 (62.66)	372 (82.6)

C. coli=Campylobacter coli, C. jejuni=Campylobacter jejuni, C. upsaliensis=Campylobacter upsaliensis, C. hyointestinalis=Campylobacter hyointestinalis, C. lari=Campylobacter lari

Table-2: Sex distribution of *Campylobacter* isolates in pigs fecal samples from Zuru Kebbi State.

Sex	**Positive (%)**	**Negative (%)**	**Total (%)**
Male	144 (90.00)	16 (10.00)	160 (53.33)
Female	134 (95.71)	6 (4.28)	140 (46.67)
Total	278 (92.67)	22 (7.33)	300

$\chi^2=2.796$, $p=0.0945$, $p>0.05$

Table-3: Age distribution of *Campylobacter* isolates in pigs fecal samples from Zuru Kebbi State.

Age	**Positive (%)**	**Negative (%)**	**Total (%)**
Adult pigs	137 (94.48)	8 (5.51)	145 (48.33)
Young pigs	141 (90.79)	14 (9.03)	155 (51.67)
Total	278 (92.67)	22 (7.33)	300

$\chi^2=0.8940$, $p=0.3444$, $p>0.05$

Table-4: Age distribution of *Campylobacter* isolates in human fecal samples from Zuru Kebbi State.

Age (years)	**Positive (%)**	**Negative (%)**	**Total (%)**
0-9	25 (56.82)	19 (43.18)	44 (29.33)
10-29	44 (61.11)	28 (38.88)	72 (48.00)
>30	25 (73.53)	9 (26.47)	34 (22.66)
Total	94 (62.67)	56 (37.33)	150

$\chi^2=2.171$, $p=0.2963$, $p>0.05$

Table-5: Sex distribution of *Campylobacter* isolates in human fecal samples from Zuru Kebbi State.

Sex	**Positive (%)**	**Negative (%)**	**Total (%)**
Male	48 (60.76)	31 (39.24)	79 (52.67)
Female	46 (64.79)	25 (35.21)	71 (47.33)
Total	94 (62.67)	56 (37.33)	150

$\chi^2=0.02615$, $p=0.715$, $p>0.05$

Discussions

The presence of *Campylobacter species* in pigs and human fecal samples has been established in this study. The prevalence of 92.66% from pigs is consistent with previous studies that reported a 90% [10] and 99% [21] prevalence in swine fecal material. This corroborate the fact that *Campylobacter* are swine intestinal commensals [22], as *Campylobacter* has been found to be isolated from almost all the fecal sample of pigs and only on rare occasions were *Campylobacter* not isolated and the reasons may be due to collection, transportation, storage and isolation techniques [23]. Also, contrary to an isolation rate of 5-20% reported by Coker *et al.* [3] in Lagos State, Nigeria, a higher prevalence of 62.66% was recorded from human fecal sample in this study. Coker's work was a hospital laboratory-based screening of pathogens from patients presented with diarrhea. Diarrhea in human could be of many causes [24]. However, humans sampled in this study were those who reared pigs in their houses and as such in very high proximity with pigs and this could have resulted in the difference in prevalence.

The most encountered *Campylobacter species* in this study is *C. coli,* both from pigs and humans. This agreed with previous reports [23], that *C. coli* is more adapted to swine intestine [1,25,26] and swine production environment and have been isolated from pigs on farm up to 100% of fecal samples [27]. This could also suggest that *C. coli* is a normal gut inhabitant of pigs, and that it exists in large amounts [22], or it is also possible that *C. coli* are more easily detected in pigs' feces than any other *Campylobacter* species [7,21]. A higher prevalence of *C. coli* isolated from human fecal sample contrasts previous studies where *C. jejuni* was predominant species isolated from humans [3]. The humans sampled in this study might have been infected with more of the *C. coli* from the pigs reared within their area of habitation. Also, *C. jejuni* is more isolated from clinical cases [1].

Prevalence of *C. jejuni* was also high when compared with previous studies [23] in which it was rarely

isolated. The higher rate of isolation in pigs here might be due to contamination from humans and other animal species such as poultry and wild birds. Other species: *C. upsaliensis* and *C. hyointestinalis* had a low isolation rate, this could be due to the fact they are less frequently encountered in pigs and man. The lower isolation rate of these species of *Campylobacter* may be attributed to the incubation temperatures of 42°C used in this study which optimized the growth of thermophilic *Campylobacters: C. coli* and *C. jejuni,* which are of public health importance [8, 28]. *C. lari* was not isolated in the fecal sample of pigs; this is not strange as *C. lari* is not usually associated with pigs [7].

Both age and sex had no significant effect on the prevalence of *Campylobacter* species both in pigs and humans. However, in humans, contrary to previous works where higher isolate were gotten from children <10 years old and the elderly [3], higher isolates were observed between the ages of 10 and 29 years in this study. This might be because in the study area, people from this age group usually have more contact with pigs and other domestic animals such as poultry, than other age groups.

In conclusion, most pigs and humans found in the pig rearing areas of Zuru Kebbi State harbored *Campylobacter* species at a very high prevalence of 92.67% and 62.67% from pigs and humans respectively. We, therefore recommended that pig farming should be intensive and sited far away from human dwellings, with maximum biosecurity and bio-safety activities being put in place. Hands should be thoroughly and frequently washed with soaps and water after contact with pigs, pets and other farm animals. Also, the use of molecular techniques may increase the diagnostic efficiency of *Campylobacter* species in both humans and pigs from the study area.

Authors' Contributions

This study was part of PBG's MPVH dissertation. MDS designed the study, OOF, AAM and MBA supervised and approved the study and experimental protocol. EBI and ION performed the statistical analysis and drafted the manuscript while OOF and MDS critically reviewed the manuscript. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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