Nigerian Veterinary Journal
VOL:32 (4) 349-356
ARTICLE

Prevalence and Molecular Characterization of Thermophilic *Campylobacter* Species Isolated From Cattle in Plateau State, Nigeria

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SUMMARY

A study was designed to determine the prevalence of *Campylobacter* species isolated from cattle in Plateau state, Nigeria. From April, 2008 – March, 2009, 352 rectal swab samples were randomly taken from cattle in 18 herds in Plateau State, Nigeria and analyzed for the presence of *Campylobacter* species. Out of the 352 samples tested, 65 (18.5%) were identified as *Campylobacter* species using biochemical tests; with 52 (80%) as *C. jejuni* and 13 (20%) as *C. coli*. Of the 65 isolates, 63 (97%) were confirmed by polymerase chain reaction (PCR). Out of the 18 cattle herds tested, 12 (66.7%) were positive. The results of the study also showed that the prevalence was significantly ($p<0.05$) higher in calves (25%) than in adults (12.2%).

KEY WORDS: Prevalence, thermophilic Campylobacter, PCR, cattle

INTRODUCTION

Thermophilic *Campylobacter* species, in particular *Campylobacter jejuni* and *Campylobacter coli,* are among the most frequently identified pathogens found to be causing gastroenteritis in humans (WHO, 2001). Chickens and cattle are the principal sources of *C. jejuni* pathogenic to humans, whereas wild animal and environmental sources are responsible for just 3% of the disease (Wilson et al., 2008). The routes of transmission of *Campylobacter* between food animals and humans are several and complex; however, the foodborne transmission route is the mode by which majority of the cases have been reported (Andersen *et al.,* 2006). Raw poultry meat has often been suggested as the major source of human campylobacteriosis because of frequent occurrence in poultry meat (Wingstrand *et al.*, 2006). Poultry is not the only sources of human infections as it has been documented that carriers of zoonotic campylobacters are common among other animal species (Petersen *et al.*, 2001).

Cattle have been incriminated in relation to outbreaks and sporadic cases mainly through the consumption of unpasteurized milk (Evans *et al.,* 1996; Sato *et al.,* 2004), consumption of beef products (Osano and Arimi, 1999), contaminated surface water run-off from cattle pastures and by direct contact with cattle (Gilpin *et al.*, 2008). The bovine reservoirs are also important for the spread of campylobacters to water and the environment during disposal of abattoir effluents and slurries which subsequently find their way back to humans through drinking of untreated water (Tauxe, 1992).

In Nigeria, limited information is available on the prevalence of *Campylobacter* species in animals especially, cattle

(Adetosoye and Adeniran, 1987; Ngulukun *et al*., 2009). This study was therefore designed to determine the prevalence of thermophilic *Campylobacter*strains isolated from cattle in Plateau State, Nigeria.

MATERIALS AND METHODS

Study area and sampling

Plateau State is located in north central Nigeria. It is divided into 3 Agricultural zones (North, Central and South) with an average of 6 local government areas (LGA) in each zone. From each of the agricultural zones of the state, 2 local government areas were selected by simple balloting and from each of the LGA selected, 3 cattle herds were randomly selected. A cattle herd consist of about 60 - 100 animals. In each of the cattle herd selected, 10 adult (> 1 year old) and 10 calves (< 1 year old) were selected by random sampling. In herds with 10 or less calves, all were selected. Rectal swab was collected from the animals selected using sterile swab sticks and put in Amies transport medium (Oxoid) and transported on ice to the laboratory. The samples were cultured immediately on reaching the laboratory. The sex and age of the animals sampled were recorded. A total of 352 rectal swab samples were collected from 18 cattle herds from April, 2008 to March, 2009.

Isolation and identification of *Campylobacter* strains

The rectal swab samples were enriched in 9 ml of Bolton broth (Oxoid) and incubated at 42°C for 24 h under a micro-aerobic condition achieved by Campy Gen^{R} (Oxoid) in an anaerobic jar. One loopful (10 ul) of enrichment culture was spread onto modified *Campylobacter* charcoal differential agar (mCCDA) plates (Oxoid), which were incubated under the same conditions. Two to three suspect colonies of campylobacter from mCCDA plates originating from enrichment procedures were subcultured onto blood agar (BBL, Becton Dickinson, MD). Two to three colonies from blood agar were identified to the species level using microscopic examination of motility and cell morphology, catalase and oxidase reactions, hippurate hydrolysis, and susceptibility to nalidixic acid (OIE, 2004). Nalidixic acid-resistant isolates were further examined for indoxyl acetate hydrolysis and susceptibility to cephalotin (OIE, 2004). All the isolates were stored in BHI broth supplemented with 15% glycerol at -70°C for further tests.

Confirmation of *Campylobacter* isolates using Polymerase Chain Reaction (PCR)

Genomic DNA from the *Campylobacter* isolates as well as from the reference stains (*C. jejuni ATCC* 33560 and *C. coli ATCC* 33559) were extracted using the DNeasy extraction kit (Qiagen, Germany) according to the manufacturer's protocol. Multiplex PCR assay was used to identify and confirm the *Campylobacter* species. Primers specific for *C jejuni*, *C coli* and *C lari* as described by Wang et al. (2002) were used. The PCR amplification was performed in a final volume of 25 ul containing 2.5 ul of 10X reaction buffer (100 mM KCL, 500 mM Tris-HCL{pH 8.3}, 100 mM KCL, and 50 mM $[NH_4]2SO_{4);}$ 20 mM $MgCL_2$, 200 uM of deoxynucloeside triphosphate, 1.25 U of FastStart *Tag* DNA polymerase (Roche Diagnostics, GmbH, Mannheim, Germany); 0.5 uM of C. *jejuni* and *C. lari* primers; 1 uM of *C. coli* primer (Integrated DNA Technologies, Cape Town, South Africa) and 2.5 ul of template DNA. The DNA amplification was carried out in a Perkin - Elmer thermocycler using an initial denaturation step at 95 ^{O}C for 6 minutes followed by 30 cycles of amplification (denaturation at 95 ^{O}C for 30 seconds followed by annealing at 59 ^{O}C for

30 seconds and extension at 72 °C for 30 seconds), ending with a final extension at 72 °C for 7 minutes. The PCR product was maintained at 4 °C until analysed.

The PCR amplified products were separated by subjecting 5 ul aliquots mixed with the loading dye to 1.5% agarose gel electrophoresis (Wang et al., 2002) for 45 minutes at 100 V and visualized using the gel documentation chamber. The desired product is at 323 bp, 126 bp and 251 bp for *C. jejuni*, *C. coli* and *C. lari* respectively. A 50 bp DNA ladder was used as a size marker.

Statistical analysis

The significance of association in prevalence of Campylobacter infection between calves and adults were analysed using the chi-square test. A probability value of < 0.05 was considered statistically significant.

RESULTS

Prevalence and distribution of Campylobacter species

Of the 352 samples tested, 65 (18.5%) were positive for *Campylobacter* species with 52 (80%) as *C. jejuni* and 13 (20%) as *C. coli* (Table 1). Out of the 18 cattleherds tested, 12 (66.7%) were positive for *Campylobacter* species. The herd prevalence ranged from 0 – 60% (Table 3). Four (22.2%) of the herds had prevalence between 40 – 60% while 8 (44.4%) had prevalence between 10 – 30%. The prevalence was significantly ($P < 0.05$) higher in calves (25%) than in adults (12.2%) [Table 1]. The PCR amplification generated 126 bp and 323 bp DNA fragments corresponding to *Campylobacter coli* and *Campylobacter jejuni* respectively (Fig. 1).Out of the 65 *Campylobacter* isolates tested using PCR, 63 (96.9%) were correctly confirmed as *C. jejuni* or *C. coli*. One isolate diagnosed by biochemical test as *C. coli* was identified as *C. jejuni* by PCR while one was negative.

DISCUSSION

In this study, the overall prevalence of thermophilic *Campylobacter* species in cattle was 18.5%. This is higher than 15% (Olubunmi and Adeniran, 1986) and 10% (Adetosoye and Adeniran, 1987) earlier reported in Southwestern Nigeria. This is also higher than 12.7% reported in cattle in Ethiopia (Kassa *et al.*, 2007). The result was however different from that reported by Adegbola *et al.* (1991) who failed to isolate any *Campylobacter* organism from apparently healthy cattle in Ogun State, Nigeria. However, higher prevalence rates have been reported in other countries, 23% in Denmark (Mazick *et al.*, 2006), 30.5% in Norway (Johnsen et al., 2006), 53.9% in Italy (Pezzotti et al., 2003), 34.1% in USA (Bae *et al.*, 2005), 31.1% in Finland (Hakkinen et al., 2007), and 62% in UK (Atabay and Corry, 1998). In the present study, 66.7% of the herds were colonized with thermophilic *Campylobacter* species. This is however lower than 83.3% reported in dairy herds in Denmark (Nielsen, 2002). The predominant *Campylobacter* species in the study area in cattle was *C. jejuni* followed by *C. coli*. Other thermophilic *Campylobacter* species were not isolated. This is in agreement with other studies (Nielsen, 2002; Bae *et al.*, 2005; Kassa *et al.*, 2007). In this study, the prevalence of thermophilic *Campylobacter* species was significantly higher in calves than adults, which is similar to other studies (Nielsen, 2002; Sato *et al.*, 2004; Johnsen *et al.*, 2006; Kassa *et al.*, 2007; Hakkinen et al., 2007). Giacoboni *et al.* (1993), in a study in Japan reported that 97% of calves less than a year old and 47% of older cattle were infected with campylobacter species which is comparable to our study where we found 66.2% in calves and 33.8% in adults. *Campylobacter* was isolated from cattle in all the local government areas studied. There was no significant difference in the prevalence of *Campylobacter* species in the 3 agricultural zones of the state. This shows that *Campylobacter* infection is widely distributed in the state. There was also no significant difference between the male and female carriers. The role of

ruminants as reservoirs of *Campylobacter* species and their potential importance as sources of human infection have been reviewed by Stanley and Jones (2003). In Newzealand, outbreaks of campylobacteriosis have been associated with people living or working on dairy farms (Gilpin *et al.*, 2008). Contact of the pastoralists and their cattle, drinking of raw unpasteurized cow milk as is the common practice in Nigeria and drinking of contaminated water from the same source with the cattle may predispose the communities to infection with campylobacters. The significance of *Campylobacter* colonization of cattle relates not only to the potential for contamination of milk at the farms and the carcass at slaughter, but also surface water during disposal of abattoir effluents and animal slurry onto land (Inglis *et al.*, 2004).

Out of the 65 *Campylobacter* isolates confirmed positive by the biochemical tests, 63 (96.9%) were confirmed as *C. jejuni* or *C. coli* by PCR. Those that were positive by biochemical tests but were negative by PCR could have been other *Campylobacte*r species other than *C. jejuni*, *C. coli* and *C. lari* or other related bacteria. The isolate identified by biochemical tests as *C. coli* but confirmed as *C. jejuni* by PCR could have been *C. jejuni* that do not hydrolyse sodium hippurate and were misidenfied as *C. coli* on the basis of the hippurate test.

CONCLUSION

Our findings revealed that *Campylobacter jejuni* and *Campylobacter coli* are prevalent in cattle in Plateau state, Nigeria. PCR confirmed *Campylobacter jejuni* and *Campylobacter coli* isolates. This report provides current information on the prevalence of thermophilic *Campylobacter* strains isolated from cattle in Plateau State, Nigeria and highlights the public health significance of this important pathogen. Further studies are needed to improve the surveillance of *Campylobacter* in food animals in Nigeria.

ACKNOWLEDGEMENTS

The authors wish to thank the cattle farmers for allowing us to carry out this research work on their farms. We also wish to acknowledge the contributions of Mr Bitrus Yakubu and Dr Jide Owolodun of the Molecular Biology Laboratory, National Veterinary Research Institute, Vom, Nigeria for their technical advice. We are also grateful to the members of the *Campylobacter* Research Group, N.V.R.I, Vom.

Table 1.Prevalence and age distribution of *Campylobacter* spp. in cattle in Plateau state.

LGA	Age group	No tested No	(%) positive	*C. jejuni*	*C. coli*
JS	Calves	30	10 (33.3)	9 (90)	1 (10)
	Adults	30	3 (10.0)	3 (100)	0 (0)
BA	Calves	28	7 (25.0)	7 (100)	0 (0)
	Adults	30	2 (6.7)	1 (50)	1 (50)
MG	Calves	29	5 (17.2)	3 (60)	2 (40)
	Adults	30	1 (3.3)	0 (0)	1 (100)
BK	Calves	26	8 (30.8)	5 (62.5)	3 (37.5)
	Adults	30	10 (33.3)	9 (90)	1 (10)
QP	Calves	29	6 (20.7)	6 (100)	0 (0)
	Adults	30	2 (6.7)	2 (100)	0 (0)
LG	Calves	30	7 (23.3)	5 (71.4)	2 (28.6)
	Adults	30	4 (13.3)	2 (50)	2 (50)
Total	**Calves**	**172**	**43 (25.0)***	**35 (81.4)**	**7 (18.6)**
	Adults	**180**	**22 (12.2)**	**17 (77.3)**	**6 (22.7)**
	Total	**352**	**65 (18.5)**	**52 (80)**	**13 (20)**

JS = Jos south LGA, BA = Bassa LGA, MG = Mangu LGA, BK = Bokkos LGA, QP = Quan-Pan LGA, LG = Langtang North LGA, * significant difference

Table 2. Herd prevalence of *Campylobacter* species in cattle

Herd	No. tested	No. positive	%
JSH1	20	5	25
JSH2	20	6	30
JSH3	20	2	10
BAH1	18	0	0
BAH2	20	0	0
BAH3	20	9	45
MGH1	20	4	20
MGH2	19	2	10
MGH3	20	0	0
BKH1	20	12	60
BKH2	18	4	20
BKH3	18	2	10
QPH1	19	0	0
QPH2	20	0	0
QPH3	20	8	40
LGH1	20	0	0
LGH2	20	8	40
LGH3	20	3	15

Herd prevalence: 12/18 = 66.7%
JS = Jos south LGA, BA = Bassa LGA, MG = Mangu LGA, BK = Bokkos LGA, QP = Qwan-Pan LGA, LG = Langtang North LGA, H = Herd, F = Flock

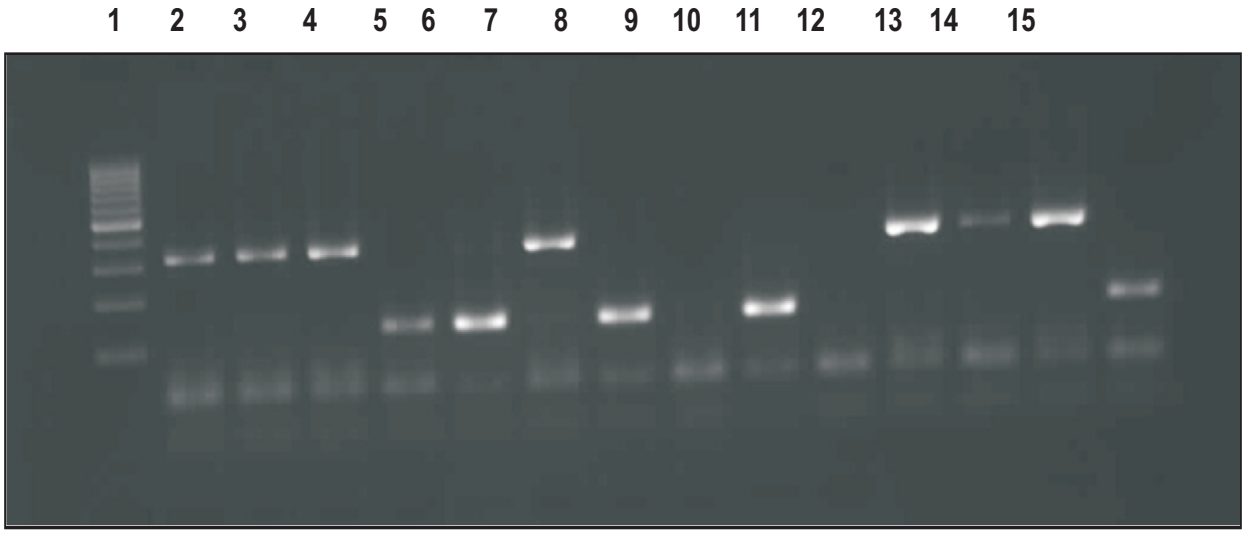

Fig. 1 Agarose gel showing amplification products of *C. jejuni* and *C. Coli* isolated from cattle in Plateau state, Nigeria. Lane 1 (molecular marker, 50 bp), lanes 2, 3, 4, 12, 13 and 14 (*C. jejuni*), lanes 5, 6, 10 and 15 (*C. coli*), lane 11 (negative sample), lane 7 (positive control, *C. jejuni* ATCC 33560), lane 8 (positive control, *C. coli* ATCC 33559), lane 9 (negative control).

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