Isolation and prevalence of *Campylobacter* species in cattle from Sokoto State, Nigeria

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Summary

The prevalence of *Campylobacter* spp. in cattle in Sokoto State, Nigeria, was determined. The number of samples collected totalled 976, of which 126 (12.9%) yielded *Campylobacter* spp. The species of *Campylobacter* isolates from this study were as follows: *C. jejuni* (65.1%), *C. coli* (23.0%), *C. lari* (7.9%), *C. hyointestinalis* (3.2%) and *C. fetus* (0.8%). A total of 172 strains of *Campylobacter* spp. were identified from the positive samples due to identification of more than a single strain (spp.) from a single sample. The strains identified were C. *jejuni* (62.8%), *C. coli* (25.0%), *C. lari* (8.1%), *C. hyointestinalis* (2.9%) and *C. fetus* (1.2%). More than one species of *Campylobacter* was identified in 36.5% of the positive samples. The biotyping in this study revealed *C. jejuni* biotype I (34.3%) as the most common *C. jejuni* biotype, while *C. jejuni* biotype IV (15.7%) was the *C. jejuni* biotype that was least frequently isolated. However, the most frequently isolated *C. coli* biotype was biotype I (72.1%) and all the isolates of *C. lari* were biotype I.

Keywords

Biotype, *Campylobacter*, Cattle, Isolation, Nigeria, Prevalence, Public health, Sokoto.

Isolamento e prevalenza di *Campylobacter* in allevamenti bovini nello stato di Sokoto in Nigeria

Riassunto

Nel presente lavoro viene esaminata la prevalenza di Campylobacter *spp. in allevamenti bovini nello stato di Sokoto in Nigeria. Su 976 campioni, 126 (12,9%) sono risultati positivi per* Campylobacter *spp. Sono stati isolati:* C. jejuni *(65,1%),* C. coli *(23,0%),* C. lari *(7,9%),* C. hyointestinalis *(3,2%) e* C. fetus *(0,8%). Sono stati identificati 172 ceppi di* Campylobacter *spp. da campioni positivi e più ceppi (spp.) da un singolo campione. I ceppi identificati sono stati* C. jejuni *(62,8%),* C. coli *(25,0%),* C. lari *(8,1%),* C. hyointestinalis *(2,9%) e* C. fetus *(1,2%). Nel 35,6% di campioni positivi è stata identificata più di una specie di* Campylobacter. *La biotipizzazione ha evidenziato tra i più diffusi* C. jejuni *biotipo I (34,3%) e* C. jejuni *biotipo IV (15,7%), il* C. jejuni *biotipo è risultato quello isolato con meno frequenza. Il biotipo di* C. coli *isolato con più frequenza è stato il biotipo I (72,1%). Tutti gli isolamenti di* C. lari *sono risultati del biotipo I.*

Parole chiave

Biotipo, Bovino, *Campylobacter*, Isolamento, Nigeria, Prevalenza, Salute pubblica, Sokoto.

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Introduction

Campylobacter species are common bacterial pathogens that cause gastroenteritis in humans, both in industrialised and developing countries (9, 32, 38). Members of the genus *Campylobacter* have long been recognised as a cause of septic abortion in both cattle and sheep (4), but the development and improvement of *Campylobacter* selective culture media lead to the recognition that *Campylobacter* can be an aetiological agent of human gastroenteritis (34).

A variety of *Campylobacter* subspecies have been isolated from healthy and diseased cattle (3). Campylobacters have been reported to cause enteritis in calves (1, 2). *Campylobacter jejuni* ssp. *jejuni* and *C. hyointestinalis* have occasionally been reported to cause bovine abortion (12, 36) and *C. jejuni* ssp. *jejuni* has been isolated in bovine mastitis (29). *Campylobacter* species have been isolated from the faeces of healthy cattle (3, 5, 18, 19, 20, 24). Although the chicken is the species most frequently identified as a reservoir of bacteria responsible for human infection, major outbreaks have been recorded from contaminated or inadequately pasteurised milk (16, 28, 31). Studies have reported an association between *Campylobacter* infection in humans and contact with cattle (3, 18, 23, 25, 30, 35).

Direct-contact exposure to bovine faeces and ingestion of unpasteurised bovine milk are well documented causes of outbreaks of campylobacteriosis (15, 33). A high degree of genetic relatedness between the *Campylobacter* from cattle and humans in the same geographical area has been reported by Fitzgerald *et al.* (17). Given the potential linkage between *Campylobacter* spp. harboured by cattle and human disease, the present study was conducted to determine the prevalence and distribution of a variety of *Campylobacter* spp. in cattle in Sokoto State.

Materials and methods

The study was conducted between November 2007 and January 2009. The prevalence of the infection was estimated on the basis of expected prevalence of 50%, with 5% as the maximum acceptable error and 95% the confidence interval. The choice of 50% expected prevalence was due to a lack of knowledge of the true prevalence of *Campylobacter* infection in cattle in the state (Sokoto) and the country (Nigeria). A total of 976 rectal swabs were collected from cattle across the state and transported to the laboratory within 6 h of the time of sampling. At the laboratory, each individual swab was inoculated into modified charcoal cefoperazone deoxycholate agar (mCCDA) medium (Oxoid, CM739) supplemented with cefoperazone and amphotericin B (Oxoid, SR155) for selective isolation of *Campylobacter* spp. at 42°C for 48 h to 96 h in an anaerobic jar containing a microaerophilic generating sachet (Campygen) (Oxoid, CN35A).

From each plate, up to five colonies with colonial morphology consistent with thermophilic *Campylobacter* spp. were streaked for isolation on blood agar and incubated for 24 h at 42°C. Presumptive thermophilic *Campylobacter* colonies were then suspended in proteose peptone glycerol (10%) and stored at –70°C for subsequent species identification and biotyping.

Campylobacter isolates were cultured on Columbia agar plates containing 5% sheep blood in a microaerophilic condition (Campygen, Oxoid, CN35A). All isolates were characterised using the standard *Campylobacter* phenotypic identification procedure described by Atabay and Corry (3). Biotyping of the isolates was performed using the extended biotyping scheme described by Lior (27).

Results

The major phenotypic characteristics of the isolates in this study were typical of thermophilic *Campylobacter* spp. All the isolates demonstrated spiral or curved rod morphology in Gram staining, the isolates revealed oxidase and catalase activity and reduced nitrate. Hippurate was hydrolysed by suspected *C. jejuni* isolates.

A total of 126 samples (12.9%) were positive for *Campylobacter* isolations. *C. jejuni* was isolated in 82 (65.1%) of the positive samples, *C. coli* in 29 samples (23.0%) and *C. lari* in 10 (7.9%) of the positive samples. *C. hyointestinalis* and *C. fetus* were isolated in 4 (3.2%) and 1 (0.8%) of the positive samples, respectively, as shown in Table I. Overall, 172 strains of the *Campylobacter* spp. were identified due to isolation of more than a single strain (species) from a single sample. Approximately 36.5% of the positive samples yielded more than a single species of *Campylobacter*. The strains identified were as follows (Table I):

- C. *jejuni* 108 (62.8%)
- *C. coli* 43 (25.0%)
- *C. lari* 14 (8.1%)
- *C. hyointestinalis* 5 (2.9%)
- *C. fetus* 2 (1.2%).

Table I
Percentage isolation of *Campylobacter* spp.

Species	No. positive (%)	No. of strains isolated (%)
Campylobacter jejuni	82 (65.1%)	108 (62.8%)
Campylobacter coli	29 (23.0%)	43 (25.0%)
Campylobacter lari	10 (7.9%)	14 (8.1%)
Campylobacter hyointestinalis	4 (3.2%)	5 (2.9%)
Campylobacter fetus	1 (0.8%)	2 (1.2%)

The isolates were biotyped as described by Lior (27); the biotyping revealed that *C. jejuni* biotype I 37 (34.3% was the most common *C. jejuni* biotype in this study, while *C. jejuni* biotype IV 17 (15.7%) was the least frequently *C. jejuni* biotype isolated. However, the most frequently isolated *C. coli* biotype was biotype I 31 (72.1%); all isolates of *C. lari* were biotype I (Table II).

Discussion

The occurrence of human *Campylobacter* gastroenteritis has largely been attributed to the consumption of contaminated food animal products (4), especially poultry, because of the high prevalence of *Campylobacter* in these animals (10, 11, 22). There is evidence that suggests other vehicles such as red meat, environmental water and unpasteurised milk as important sources of these organisms (21, 33).

Table II
Biotypes of the thermophilic *Campylobacter* isolates

Campylobacter	Biotypes	Isolation rate (%)
Campylobacter jejuni	I	37 (34.3%)
	II	26 (24.1%)
	III	28 (25.9%)
	IV	17 (15.7%)
Campylobacter coli	I	31 (72.1%)
	II	12 (27.9%)
Campylobacter lari	I	14 (100%)
	II	0 (00.0%)

The isolation conditions in this study were developed for the isolation of thermophilic campylobacters (*C. jejuni*, *C. coli* and *C. lari*). This may bias detection in favour of thermophilic campylobacters (3, 4, 6, 14). The prevalence of thermophilic campylobacters in this study was 12.9% and is in agreement with previous studies in which prevalence of campylobacters in cattle ranged from 0.8% to 46.7%, depending on the method of isolation, season, age of animal and sample size (4, 8, 19, 23, 32, 37).

The concurrent excretion frequency in this study (36.5%) is higher than the 9% rate reported by Bae *et al*. (4) and 24% reported by Inglis *et al.* (25). Considering the composition of isolation media and the isolation conditions in this study, the isolation of *C. hyointestinalis* is however possible but was isolated at a lower rate in this study. This is in agreement with the observations of Atabay and Corry (3), Busato *et al.* (7), Giacoboni *et al.* (19) and Inglis *et al.* (25). The isolation of thermotolerant *C. fetus* in this study was fortuitous; some investigators have reported the isolation of similar atypical *C. fetus* strains from raw milk and humans (13, 26, 39).

The most common biotype of *C. jejuni* in this study was biotype I which accounts for 34.3% of the total *C. jejuni* isolates. This observation

was in agreement with that of Baserisalehi *et al.* (5) who observed more of the *C. jejuni* biotype I than any other *C. jejuni* biotypes. The common *C. coli* biotype in this study was biotype I. The identification of these biotypes from cattle in the state is of serious public health importance, since these biotypes have been implicated as the cause of disease in humans.

The results of this study demonstrate that *C. jejuni* is widely distributed among cattle in Sokoto State.

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