Original Article

Prevalence of *Campylobacter* among goats and retail goat meat in Congo

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Abstract

Background: The prevalence of *Campylobacter jejuni* and *Campylobacter coli* was determined in goat and goat meat sold at retail outlets in Lubumbashi, Democratic Republic of Congo (DR Congo).
Methodology: A total of 644 samples, including 177 goat meat, 86 goat stomachs, 139 ready to eat (RTE) goat skewers, and 242 goat faecal samples were examined for the presence of *Campylobacter jejuni* and *Campylobacter coli* using polymerase chain reaction.
Results: Overall, *Campylobacter* spp. were found in 34.6% of the examined samples. *C. jejuni* was isolated in 10.1% and *C. coli* in 26.7% of samples. Only 2.2% of all samples were positive for both species. There was a significant association between the prevalence of *C. coli* and the type of sample ($p < 0.05$). The overall prevalence of *Campylobacter* in different sample groups was 41.2%, 37.2%, 23.7%, and 35.1% for goat meat, goat stomachs, RTE goat skewers, and goat faecal samples, respectively. There was no significant difference ($p > 0.05$) between the prevalence observed in the rainy season (16.7%) and the dry season (20.0%). Moreover, the overall prevalence of *Campylobacter* in slaughter sites, open-air markets, warehouses, and semi-open-air markets was 28.2%, 34.2%, 35.4%, and 42.9%, respectively. Statistically, there was no influence of the sample collection site on the frequency of isolation of *Campylobacter* ($p > 0.05$).
Conclusion: This study shows that, considering the relatively high prevalence of this pathogen, live goat and goat meat are major sources of human and environmental contamination by *Campylobacter* spp. in Lubumbashi.

Key words: *campylobacter jejuni*; *campylobacter coli*; goat; polymerase chain reaction; prevalence

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Introduction

Campylobacter is one of the major pathogens involved in food-borne illnesses [1,2,3] and it has been reported that only 500 cells of *C. jejuni* can cause human illness [4,5]. A report involving 111 adult volunteers showed that ingestion of 8 x10^2 to 2 x10^9 organisms caused diarrhoeal illness [6]. In addition, campylobacteriosis was identified as the predominant cause of Guillain-Barré syndrome (GBS), a demyelinating polyneuropathy [7,8,9,10]. In 2005, the European Union reported 51.6 cases of campylobacteriosis per 100,000 inhabitants. About 91% of isolates were characterized as *C. jejuni* and the rest were *C. coli* [11]. In Belgium, the incidence of campylobacteriosis was 64.5 per 100,000 inhabitants in 2006 [12].

Campylobacter spp. are normally carried in the intestinal tracts of many domestic livestock such as poultry, cattle, sheep, pigs, as well as wild animals and birds [13,14,15,2]. Humans are contaminated by ingestion of undercooked meat, or by contact with animals and contaminated surface water or unpasteurized milk and dairy products [16,17]. In 2007, The Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organization (WHO) reported an 83% prevalence of *Campylobacter* in food retail products in many European countries. In Iran and Japan, *Campylobacter* prevalence rates of 63% and 45.8%, respectively, were reported in chicken meat [18,15].

In Lubumbashi, in the south of DR Congo, goat constitutes the major source of the meat supply. Goats are usually reared in close contact with humans, along with other types of domestic pets such ash chickens, pigs and dogs, similar to husbandry systems in other African countries such as Nigeria [19]. However, in Lubumbashi, appropriate infrastructure such as slaughterhouses and butcher shops for retail meat do

not exist. Overall, the slaughtering process is performed under poor hygienic conditions. Routine practices include slaughtering animals beside the goat skewer cooking stall. Goat carcasses are hung on the stall to allow the consumer to directly select the piece of meat to be cooked. Goat meat is generally sold at retail outlets in the various markets in Lubumbashi at ambient temperature. There are no measures taken to protect the displayed food from insects, such as flies, or from dust. These facilities were considered representative of goat meat retail outlets in Lubumbashi. *Campylobacter* has been previously isolated from apparently healthy goats in Lwiro, eastern DR Congo. [20], Ethiopia [14], and Sokoto State, Nigeria [19]. This demonstrates that *Campylobacter* could be found on goat meat, as animal food products can be contaminated by this pathogen during slaughter or carcass dressing; also, cross-contamination of ready to eat (RTE) foods can occur during preparation by food handlers and by direct contact with animals and contaminated materials such as forks, knives, and water. Therefore, the improper meat handling conditions observed in Lubumbashi can constitute a real threat to public health.

In African regions, there is limited information on campylobacteriosis due to the lack of surveillance systems. The objective of this study was to determine the prevalence of *Campylobacter* in live goats and goat meat/meals in Lubumbashi.

Methodology

Sample collection

Goat meat

Sampling was carried out in the rainy and dry seasons between April 2009 and May 2010. Samples were collected from 6 open-air markets, 6 semi-open-air markets, 32 snack bars, and 17 bars in six municipalities of Lubumbashi: Kamalondo, Kampemba, Katuba, Kenya, Lubumbashi, and Rwashi.

A total of 402 samples of goat meat were collected from the sites described above. They were divided into: goat meat (n = 177), of which 154 were collected from markets and 23 were collected from slaughter sites; goat stomachs (n = 86), all collected from markets; and ready-to-eat (RTE) goat skewers (n = 139), which were all collected from slaughter sites. Goat meats were sold as unspecified retail cuts of 70-150 g. The goat stomachs were sold in pieces weighing 60-120 g rolled and tied with pieces of emptied and cleaned intestines. RTE goat skewers were generally sold in snack bars or next to bars as unspecified pieces weighing 50-70 g. In 10 cases, faecal samples were collected from live goats before slaughter, then meat samples were collected after slaughtering, and a few hours later, skewers cooked from the rest of the carcasses were collected.

All samples were transported to the laboratory in an isothermal box at 4°C and analysed within about one to four hours of collection.

Goat faecal samples

Goat faecal samples were collected in the rainy and dry seasons from four warehouses (Kabasele, Essence, Mimbulu and Ndjandja) that specialized in the sale of goats. In addition, 52 goat slaughter sites were included in the study; these sites were adjacent to the goat skewer cooking stalls. Faecal samples were harvested directly from the rectums of goats: 242 faecal samples were collected, of which 209 were taken from four warehouses and 33 from goat slaughter sites. Samples were transported in sterile tubes to the laboratory for further analysis.

Sample preparation and bacteria isolation

Isolation and characterization of *Campylobacter* spp. from goat meat

Analyses were performed at the laboratory of Expertise, Hygiene and Technology of Food from Animal Origin, University of Lubumbashi. Within two to four hours of sample collection, 25 g of each sample was mixed with 225 mL of buffered peptone water (BPW); the mixture was homogenized using a Stomacher 400 (Seward, London, UK), then incubated at 37°C for 18 hours. Volumes of 1 mL of the mixture were further enriched in Bolton broth (Oxoid, Basingstoke, UK) and incubated at 42°C for 18 to 24 hours under microaerophilic conditions. Each enriched sample was stored at -20°C for further characterization by PCR in the Laboratory of Medical Microbiology, University of Liège. The interpretation of culture and PCR results was based on the presence or absence of bacteria in the culture, or the presence or absence of its target gene in a 25 g sample analysed by PCR.

C. jejuni and *C. coli* were isolated on blood free *Campylobacter* selective agar base (Merck, Darmstadt, Germany) supplemented with charcoal cefoperazone deoxycholate agar supplement (CCDA selective supplement) (Merck) and incubated at 42°C for 48 hours under a microaerophilic atmosphere, using GENboxmicroaer atmosphere generators (bioMérieux, France). The morphology of colonies, motility, and biochemical tests such as production of oxidase (Merck, Darmstadt, Germany) and catalase (Merck,

Darmstadt, Germany) were used to confirm the presence of *Campylobacter*. The differentiation between *C. jejuni* and *C. coli* was done using the hippurate hydrolysis test (Rosco, Taastrup, Denmark) [21,16].

Isolation and characterization of *Campylobacter* spp. from goat faecal samples

One to 1.5 g of each goat faecal sample was suspended in physiologic water and incubated for one hour at room temperature, streaked onto blood free *Campylobacter* selective agar base (Merck, Darmstadt, Germany) supplemented with CCDA selective supplement (Merck, Darmstadt, Germany), then incubated at 42°C for 48 hours under microaerophilic conditions using GENboxmicroaer atmosphere generators (bioMérieux, Marcy l'Etoile, France). Presumptive colonies of *Campylobacter* were further characterized as described above.

DNA extraction

DNA was directly extracted from enriched samples in Bolton broth for meat samples or from sterile water for faecal samples. Volumes of 200 µL of each enriched sample were centrifuged and bacterial pellets were collected. For cell lysis, 100 µL of a buffer containing 50 mMKCl, 10 mMTris-HCl pH 8.3, 2.5 mM $MgCl_2$ (Applied Biosystem, Branchburg, USA), 0.5% Nonidet P40 (Roche Diagnostic, Mannheim, Germany) and 0.5% Tween 20 (Promega, Madison, USA) supplemented with 3 µL of proteinase K (20 mg/mL) (Qiagen, Hilden, Germany) were added to bacterial pellets and heated for 30 minutes at 60°C and then for 30 minutes at 100°C. Cell debris was removed by centrifugation at 13,000 rpm for five minutes using a centrifuge 5415R (Eppendorf, Hamburg, Germany). The supernatant was used as the DNA template for PCR assays.

PCR assay

Two simplex PCRs were developed to identify *C. jejuni* and *C. coli* based on *ceuE* genes present in both species. Target genes, primer sequences and amplicon sizes are listed in Table 1 [44]. The PCR experiments were performed on reference strains of *C. jejuni* N204, T276, and L359, and *C. coli* Y58, T287, and A747 provided by the Reference Laboratory of *Campylobacter* / UHC Saint Pierre (Belgium). To perform the PCR, 3 µL DNA (50-100 ng/µL) were added to a PCR Mix containing a 10X-concentrated Tris-HCl buffer, $MgCl_2$ (Applied Biosystems, Foster City, USA), 200 µM dNTPs, 2.5 U *AmpliTaq* DNA polymerase (Promega, Fitchburg, USA), and 30 pmol of each primer (Eurogentec, Seraing, Belgium), reaching a 50 µL total volume of reaction. PCR conditions were 1 minute at 94°C for DNA denaturation, 15 seconds at 52°C for annealing, and 1 minute at 72°C for elongation, in 30 cycles. PCR amplicons were resolved in a 2% agarose gel (Invitrogen, Shmona, Israel) and were visualized under UV.

Statistical analysis

Statistical analysis of the results was conducted using SPSS version 13.0 for Windows (SPSS Inc., Chicago, USA), and the methods used were the chi-squared contingency table test and Fisher's exact test to determine whether there were significant differences in the prevalence of *Campylobacter* between matrices analysed, sampling sites, and seasons. Results were considered significant at the 5% level. The concordance of results obtained by culture and by PCR was expressed using Cohen's kappa coefficient (determined with SPSS 13.0).

Results

Detection of Campylobacter by PCR

Out of 644 samples, 34.6% were found to be contaminated with *Campylobacter*; 10.1% were identified as *C. jejuni,* while 26.7% were identified as *C. coli*. The prevalence of *C. coli* was related to the sample type ($p < 0.05$), while no association was found for *C. jejuni* ($p > 0.05$). *Campylobacter* was detected in 41.2%, 37.2%, 23.7%, and 35.1%, respectively, of goat meat, goat stomachs, RTE goat skewers, and goat faecal samples. The prevalence in the rainy season was 16.7%, while the prevalence in the dry season was 20.0%, but this difference was not significant ($p > 0.05$). Concerning the sites of sample collection, the overall prevalence of *Campylobacter* of 28.2%, 34.2%, 35.4%, and 42.9% were determined, respectively, for slaughter sites, open-air markets, warehouses, and semi-open-air markets (Table 2). There were no correlations ($p > 0.05$) established between sites of sampling and the prevalence of *C. jejuni* or *C. coli*. Prevalence of *Campylobacter* on RTE goat skewers ($n = 10$) was compared to the prevalence previously determined on goat meat ($n = 10$) used to cook the skewers and on goat faecal samples ($n = 10$) taken prior to slaughter from the goats that supplied the meat.

Table 1. Target genes, primers, primer sequences, and amplicon sizes

Gene	Primer	Sequence of primer	Size of amplicon	Reference
ceuE	Cj1	CTGCTACGGTGAAAGTTTTGC	783 bp	[42]
	Cj2	GATCTTTTTGTTTTGTGC		
ceuE	CC2	GATTTTATTATTTGTAGCAGCG	645 bp	
	CC3	TCCATGCCCTAAGACTTAACG		

Table 2. Percentage of detection of *Campylobacter* in goat meat and faeces at different collection sites

Site of sampling	Matrix	No. of samples	*C. jejuni* (%)	*C. coli* (%)	(%) Positive for *C. jejuni* and *C. coli*	Total PCR (%) *Campylobacter* spp.
Open-air market	Goat meat	66	4 (6.1)	19 (28.8)	-	23 (34.8)
	Goat stomachs	39	1 (2.6)	12 (30.8)	-	13 (33.3)
	Total	**105**	**5 (4.8)**	**31 (29.5)**	**-**	**36 (34.2)**
Semi-open-air market	Goat meat	88	7 (8.0)	33 (37.5)	1 (1.1)	38 (43.1)
	Goat stomachs	47	6 (12.8)	13 (27.7)	-	19 (40.4)
	Total	**135**	**13 (9.6)**	**46 (34.0)**	**1 (0.7)**	**58 (42.9)**
*Goat slaughter sites	RTE goat skewers	139	13 (9.4)	24 (17.3)	4 (2.8)	33 (23.7)
	Goat meat	23	1 (4.3)	11 (47.8)	1 (4.3)	11 (47.8)
	Goat faeces	33	3 (9.1)	8 (24.2)	-	11 (33.3)
	Total	**195**	**17 (8.7)**	**43 (22.0)**	**5 (2.6)**	**55 (28.2)**
Warehouse						
Essence (Kamalondo municipality)	Goat faeces	55	7 (12.7)	19 (34.5)	3 (5.5)	23 (41.8)
Kabasele (Lubumbashi municipality)	Goat faeces	65	10 (15.4)	9 (13.8)	3 (4.6)	16 (24.6)
Mimbulu (Katuba municipality)	Goat faeces	64	8 (12.5)	21 (32.8)	1 (1.6)	28 (43.8)
Ndjandja (Kamalondo municipality)	Goat faeces	25	5 (20.0)	3 (12.0)	1 (4.0)	7 (28.0)
	Total	**209**	**30 (14.3)**	**52 (24.8)**	**8 (3.8)**	**74 (35.4)**

*Goat slaughter sites are equivalent to sites where goat skewers are cooked and collected

Table 3. Prevalence of *Campylobacter* spp. in goat meat and goat faeces

Food matrix	No. of samples	*C. jejuni* (%)	*C. coli* (%)	Positive (%) for *C. jejuni* and *C. coli*	Total PCR (%) for *Campylobacter* spp.
RTE goat skewers	139	13 (9.4)	24 (17.3)	4 (2.8)	33 (23.7)
Goat meat	177	12 (6.8)	63 (35.6)	2 (1.3)	73 (41.2)
Goat stomachs	86	7 (8.1)	25 (29.1)	0 (0.0)	32 (37.2)
Goat faeces	242	33 (13.6)	60 (24.8)	8 (3.3)	85 (35.1)
Total	**644**	**65 (10.1)**	**172 (26.7)**	**14 (2.2)**	**223 (34.6)**

The prevalence of *Campylobacter* spp. was significantly decreased in RTE goat skewers (20.0%), but it was higher in goat meat (50.0%) than the prevalence determined in goat faecal samples before slaughter (20.0%).

Isolation of Campylobacter by culture

The bacteriological culturing procedure identified 33.8% samples positive for *Campylobacter* spp. *C. jejuni* and *C. coli* were recovered in 10.4% and 25.4% of samples, respectively. Of all samples tested, 2.0% were positive for both *C. jejuni* and *C. coli*. Concordance between the results obtained by PCR and by cultural culture method was observed (kappa = 0.88), although the detection of *Campylobacter* was slightly higher by PCR than by culture. Results are summarized in Table 3.

Discussion

Little is known about the prevalence of *Campylobacter* in goats and goat meat in DR Congo. In this study, using PCR, we determined a 34.6% general prevalence rate of *Campylobacter*. The prevalence rate by culture method was found to be 33.8%. Our findings showed higher a prevalence of *Campylobacter* rates by PCR compared to the culture method. Many studies have demonstrated that PCR was much more sensitive than the cultural method [22,23].

C. jejuni was identified by culture in both outpatients (13.7%) and inpatients (24.0%) suffering from diarrhoea in Lwiro, in the region of Kivu, DR Congo. *C. jejuni* was also found among animals handled by the local population such as goats (13.0%), pigs (38.4%), and chickens (40.0%) [18]. In Ethiopia and Nigeria, prevalence rates of 9.4%, 7.6%, and 20.1% of *Campylobacter* spp. were reported by culture, respectively, in goat carcasses, in goat meat, and in goat faecal samples [24,19]. A PCR-based prevalence of 29% of *Campylobacter* was reported in goat faecal samples in the Venda region of South Africa [4]. Our findings are much higher in goat meat and goat faecal samples compared to these results. In Africa, although there is still a shortage of knowledge about the prevalence of *Campylobacter* spp. in goats and goat meat, this prevalence has been frequently reported in various livestock and in human. For example, it has been reported among cattle (12.6%), pigs (50%), and sheep (38.0%) in Ethiopia [9]; in sheep meat (18.0%) in Nigeria [13], in retail chicken carcasses (32%) in Senegal [25,26], and in human isolates (9.3%) in Tanzania and Uganda [27,28]. In other developing countries such as Iran, Pakistan, and Malaysia, *Campylobacter* was reported in 9.4% of goat meat, in 12% of lamb meat, in 10.9% of beef , and in 64.7% of chicken meat [29,30,31]. In the developed world, the existence of *Campylobacter* in various livestock such as poultry, pigs, cattle, sheep, as well as food products, has been established [32,12,15,33,34,35], but little is known about its existence in goats. *Campylobacter* spp. were found in 14.3% of goats in city farms in southern Germany [36]. This prevalence, however, is much lower than our findings in Lubumbashi.

Concerning goat stomachs and processed meat like RTE goat skewers, there is no data about the prevalence of *Campylobacter* in these types of samples. In Lubumbashi, goat skewers are cooked directly in stalls adjacent to slaughter sites. Although a lack of hygiene was often observed during slaughtering, evisceration, meat handling, and preparation, we could explain the low rate of isolation of *Campylobacter* in goat skewers collected from slaughter sites by the fact that the meat had been cooked. Indeed, during our investigations, we compared the prevalence of *Campylobacter* in goat faecal samples, then in meat after slaughter, and finally in RTE goat skewers from same origin and we observed that the highest prevalence was determined in goat meat (50.0%) compared with 20.0% in goat faecal samples; this could be a consequence of the poor hygiene conditions mentioned above. After cooking the meat, the prevalence significantly decreased (20.0%). This is similar to findings of very few *Campylobacter* spp. in poultry dishes in street restaurants in Senegal [37]; the authors of that study concluded that cooking seemed to be the major cause of destruction of *Campylobacter* and the presence of this pathogen in poultry dishes could be explained by recontamination of cooked food by raw material. In the developed world, a decrease in prevalence of *Campylobacter* spp. in RTE products was also observed. In RTE pork and RTE poultry, this prevalence was 0.1% and 0.07%, respectively, while the prevalence was 2.7% and 35.1% in raw pork and raw poultry, respectively [38].

In our study, *C. coli* was the detected more frequently (26.7%) than *C. jejuni* (10.1%). Our findings are different from results previously reported in other African regions where *C. jejuni* was the most frequently recovered bacteria [14,19,24]. In another study conducted in South Africa on diarrhoeic goats, *C. jejuni* was the most recovered bacteria (56.9%) compared to *C. coli* (13.9%), while in non-diarrhoeic

faecal samples, *C. coli* was found more often (25.9%) than *C. jejuni* (3.4%) [4]. We found similar numbers in apparently healthy goats. Other studies in Africa have reported higher rates of *C. coli* isolation. For instance, in Yaoundé (Cameroon), *C. coli* was the most recovered (68.9%) from chicken carcasses, compared to *C. jejuni* (31.1%) [7]. Similar findings were reported in sheep in Nigeria, were *C. coli* represented 72.7% and *C. jejuni* represented 44.6% of the infections [13]. This may corroborate the suggestion that *C. jejuni* is the most isolated in the developed world while *C. coli* is predominant in the developing world [39].

Seasonal variation of the prevalence of *Campylobacter* in Africa had not previously been recognized. In our study, we did not find significant differences between the rainy season (16.7%) and the dry season (20.0%). Similar results were reported in Nigeria, where *Campylobacter* spp. were isolated from 2.1% of milk samples in the cold dry season, from 6.1% in the hot dry season, and from 6.0% in the rainy season. There was no association between the seasons and the infection rate, although milk collected in the hot dry and the wet seasons showed the highest prevalence rate [40]. In the developed world, a seasonal influence on the prevalence of *Campylobacter* was reported, with peak incidences in late spring/early summer [15]. In developing countries, lack of seasonal peaks of campylobacteriosis could be explained by the lack of extreme temperature variations; outbreaks of infections are uncommon and illness lacks the marked seasonal nature. In addition, there is no adequate surveillance of epidemics [41,42].

Matrices analysed in this study were collected from slaughter sites, open-air markets, warehouses, and semi-open-air markets, but there was statistically no influence of the site of sample collection on the frequency of detection of *Campylobacter* ($p > 0.05$), although higher prevalence was determined in semi-open-air markets. In Ethiopia, goat meat collected from slaughterhouses and butcher shops showed the same prevalence of *Campylobacter* spp. (9.0%), but no *Campylobacter* spp. was found in goat meat from supermarkets [14]. In contrast, the increased prevalence of *Campylobacter* spp. in samples and sites of sample collection was previously reported on fresh and frozen chicken carcasses ($p < 0.1$). Indeed, the highest prevalence was found in samples from street vendors (50%), followed by supermarkets (48.9%) and butcheries (6.7%) [43].

Conclusion

In this study, we demonstrated that live goat and goat meat can act as major sources of human and environmental contamination by *Campylobacter* spp. in Lubumbashi. Contamination of goat meat and RTE goat skewers indicates the need to apply good hygiene practices in the slaughtering process and in meat handling. The lack of hygiene in meat handling at the sale, cooking points, and slaughter sites contributed to increased cross-contamination through live animals, meat handling, slaughtering, and cooking equipment.

Although the effectiveness of molecular methods such as PCR in the definitive identification of *Campylobacter* species, PCR has not yet been applied in food laboratories in Lubumbashi. Thus, we can recommend to public health officials to include this technique as an alternative or a complement to traditional culture methods.

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