Original article

Prevalence of Thermophilic *Campylobacter* species in carcasses from sheep and goats in an abattoir in Debre Zeit area, Ethiopia

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Abstract

Background: Thermophilic *Campylobacter* spp. namely, *Campylobacter jejuni* and *coli* cause acute diarrheal diseases in humans worldwide; although these species are known to occur in the intestinal tract of a wide variety of domestic and wild animals.

Objective: Little is known about the presence of these bacteria in various food animals as possible sources of infection to humans in Ethiopia. Therefore this study was undertaken to determine the prevalence of thermophilic *Campylobacter species* in sheep and goat carcasses at a private export abattoir in Debre-Zeit, Ethiopia.

Methods: A cross-sectional study was conducted on apparently healthy sheep and goat slaughtered at a private export abattoir in Debre-Zeit, from October 2007 to March 2008. Sheep carcasses (mutton) (n=218) and goat carcasses (n=180) were analyzed for *Campylobacter* spp. Swabs were taken from four different sites on the carcasses (crutch, abdomen, thorax and breast) at different stages of slaughtering processes (before evisceration, after evisceration and after washing).

Results: *Campylobacter* spp were isolated from 40 (10.1%) out of 398 carcasses examined. There was no statistically significant difference in the rate of isolation of *Campylobacter* species in different swabbing sites. Of the 40 thermophilic campylobacter isolates, *C. jejuni* and *C. coli* accounted for 29 (72.5%) and 11 (27.5%), respectively.

Conclusions: The results of this study revealed the presence of campylobacter in sheep and goat carcasses, indicating possible risks of infection to people through the consumption of raw/under-cooked meat. Coordinated actions are needed to reduce or eliminate the risks posed by this organism at various stages of slaughtering process. [*Ethiop. J. Health Dev.* 2009;23(3):229-233]

Introduction

The genus *Campylobacter* is of great importance in human medicine and food safety, in addition to its veterinary importance (1). The organisms can be found in the intestine of poultry, pigs, sheep, cattle and other food animals and in bulk milk samples, tissue specimens from beef cattle and raw ground beef (2). Several species of birds and rodents may act as reservoirs and are the ultimate sources for most human campylobacter infections (3). Occupational exposure may also cause infection and disease on workers in animal health facilities, animal shelters, and poultry processing plants, animal agriculture and rendering-plants (4). *Campylobacter* enteritis in man has been diagnosed with increasing frequency over recent years, mainly due to *C. jejuni, C. coli and C. lari,* and most importantly in the developing world *C. upsaliensis* is common (3, 5, 6). In a small percentage of cases, long-term and potentially serious complications can arise such as Guillain–Barré syndrome (7), bacteraemia and reactive arthritis (8). Treatment with antibiotics for uncomplicated campylobacter infection is rarely indicated. However, antimicrobial resistance to clinically important drugs used for treatment (especially macrolides and fluoroquinolones) is increasingly reported for campylobacters (9). There is growing scientific evidence that the use of antibiotics in food animals, particularly in developed countries, leads to the development of resistant pathogenic bacteria that can reach humans though the food chain (10). In Ethiopia, there are few studies regarding campylobacteriosis in humans (11, 12), food animals (13) and foods of animal origin (14) including their sero-typing (15) and antimicrobial susceptibility pattern (16-18). In order to gather more information on the presence of campylobacter in animal food products, a cross sectional study was conducted on a selected private export abattoir in Debre-Zeit, from October 2007 to March 2008.

Methods

Study Area

A cross-sectional study was carried out on purposively selected export abattoir located at Debre-Zeit which is 45 km south east of Addis Ababa, from October 2007 to March 2008. The export abattoir is one of the private slaughter houses established according to export standard.

Collection and Handling of Samples

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Sheep carcasses (mutton) (n=218) and goat carcasses (n=180) were randomly selected and sampled from four different sites (crutch, abdomen, thorax and breast) in three different operations (before evisceration, after evisceration and after washing) in each slaughtered animals. Samples were collected using standard swabbing techniques using dry sterile cotton swab and transferred into a test tube containing 10ml of peptone water (Oxoid Ltd, Basingstoke, UK). Test tubes were then put into an ice box filled with plastic ice bags and immediately transported to the Microbiology Laboratory of the Faculty-Veterinary Medicine, Addis Ababa University, Debre Zeit on the day of sample collection.

Culture and Identification of Thermo-tolerant Campylobacter Species

Approximately 10 ml of swabbed samples inoculated onto Preston Campylobacter selective agar (Oxoid) supplemented with polymyxin B, rifampicin, trimethoprim and cycloheximide (Oxoid) and 5% sheep blood. All inoculated samples were incubated at 42^{o}C for 48 hours in a microaerophilic atmosphere achieved in anaerobic jar (Oxoid) without catalyst and by using CampyGen® gas generating kits (5% O_2 and 10% CO_2). Preliminary identification of *Campylobacter* spp. was performed based on the characteristic Gram-staining reactions, positive tests for oxidase, catalase and motility reactions. Species differentiation was based on hippurate hydrolysis, and susceptibility to nalidixic acid (30 µg, Oxoid) and cephalothin (30µg, Oxoid). These parameters formed the basis for the identification of *C. jejuni*, *C. coli* or *C. lari*, as proposed by others (19). The type strains *C. jejuni* (NCTC 11351), *C. coli* (LMG 6440) and *C. lari* (NCTC 11352) were included as positive controls.

Data Management and Analysis

The data collected were entered and managed in MS Excel program. SPSS version 12 (SPSS Inc, Chicago, Illinois, USA) for windows was used for data analysis. Comparisons were made using Chi-square (χ^2) test with Yates' correction or Fisher's exact tests. A p-value of <0.05 was considered indicative of a statistically significant difference.

***Ethical Considerations*:** The research project has been approved by the Academic Commission of the Faculty of Veterinary Medicine, Addis Ababa University. Permission from the export abattoir was obtained before the commencement of the study.

Results

Prevalence

A total of 398 carcasses were investigated for thermophilic campylobacter species. Of these, 40 (10.1%) were positive for *Campylobacter* species (Table 1). The numbers and percentages of campylobacter strains isolated from sheep and goat carcasses were 23/218 (11.0%) and 17/180 (9.4%), respectively. Sheep carcass was found to be more highly contaminated than goat carcass, but no statistically significance difference was observed between the two (p=0.84). The highest rate of contamination of carcasses was observed after evisceration (6.5%) when compared before evisceration (0.8%) and after washing (3.0%) (p <0.05) (Table 1).

Table 1: **Prevalence of thermophilic *Campylobacter* species in sheep and goat carcasses in an abattoir in Debre Zeit, Ethiopia (2007-2008)**

	Proportion of positive no. (%)			
	Before evisceration	**After evisceration**	**After washing**	**Total**
Sheep carcass (n=218)	0 (0)	13 (6.0)	10 (4.5)	23 (10.6)
Goat carcass (n=180)	3 (1.7)	13 (7.2)	1 (0.6)	17 (9.4)
Total (n=398)	3 (0.8)	26 (6.5)	12 (2.8)	40 (10.0)

***Level of Contamination in Different Swabbing Sites*:** There was no statistically significant difference in the rate of isolation of *Campylobacter* spp. in different swabbing sites (crutch, 7.4% vs. abdomen 11.6%, p=0.43), (abdomen 11.6% vs. thorax 8.7%, p=0.68) and (thorax, 8.7% vs. breast, 12.6%, p =0.66) as shown in Table 2.

Table 2: **Proportion of campylobacter positive carcasses according to different swabbing sites in an abattoir in Debre Zeit, Ethiopia (2007-2008)**

	Swabbing site (no. of samples examined and no. (%) positive for campylobacter)									
	Crutch		**Abdomen**		**Thorax**		**Breast**		**Total**	
	Number examined	**No. (%)**	**Number examined**	**No. (%)**	**Number examined**	**No. (%)**	**Number examined**	**No. (%)**	**Number examined**	**No. (%)**
Sheep carcass	56	4 (7.1)	49	7 (14.3)	50	5 (10)	63	7 (11.1)	218	23 (10.6)
Goat carcass	52	4 (7.7)	46	4 (8.7)	42	3 (7.1)	40	6 (15)	280	17 (9.4)
Total	108	8 (7.4)	95	11(11.6	92	8 (8.7)	103	13 (12.6)	398	40 (10.0)

Species Differentiation

The numbers and percentage of *C. jejuni* and *C. coli* isolated from sheep and goat carcasses were presented in Table 3. Of the 40 thermophilic campylobacter isolates, *C. jejuni* and *C. coli* accounted for 29 (72.5%) and 11 (27.5%), respectively. A statistically significant difference was observed between the two *Campylobacter* species identified (p=0.005).

Table 3: ***Campylobacter*** **species distribution in sheep and goat carcasses examined in an abattoir in Debre Zeit, Ethiopia (2007-2008)**

	Campylobacter **species**		
	C. jejuni **No. (%)**	***C. coli*** **No (%)**	**Total No (%)**
Sheep carcass (n=218)	17 (7.0)	6 (2.8)	23 (10.6)
Goat carcass (n=180)	12 (6.7)	5 (2.8)	17 (9.4)
Total (n=398)	29 (7.3)	11 (2.7)	40 (10.1)

Discussion

Foods of animal origin have been incriminated as the main sources for campylobacter infection in humans (20, 21). In Ethiopia, few published reports are available regarding the occurrence of campylobacter in food animals (cattle, sheep, goats and chickens) (13) and their products (14). Raw meat particularly from beef is widely consumed in the country increasing the likelihood of pathogen transmission to humans. In the present study, the frequency of isolation of thermophilic *Campylobacter* species in sheep cascaras was 10.6% (Table 1). This is comparable to the findings reported from a previous study done in Ethiopia (10.5%) (14) and Ireland (11.8%) (22). But it is higher than the prevalence reported from Pakistan (5.1%) (23), (8.1%) from Norway (24) and (2.1%) from Australia (25). However higher prevalence has been reported in mutton (and/or lamb) with range of 15-20% elsewhere the world (26-28). In this study, the prevalence of thermophilic *Campylobacter* species in goat carcasses was 9.4% (Table 1). Different findings have been reported in different studies e.g. 6.3% in Kenya (29) and 2.7% in Canada (30).

In the present study, the highest isolation rate of *Campylobacter* was found in swabs collected from the breast region (12.6%), even though no statistically significant difference observed as compared to other sites (Table 2). The possible explanation for high level of carcass contamination in the breast region could be a contamination of carcasses with intestinal contents during manual skinning, evisceration, washing and processing in the slaughter house (31) or more frequent contact between operator's hand and the knife in the breast region. Furthermore, the breast region being a fatty tissue is more prone to lodge microorganisms when compared to lean meat (32).

Another point of contamination occurs during carcass washing. The carcass wash water harboring a large amount of microorganisms flows down the length of the carcass to the breast region resulting in heavy contamination of the breast region. The intestinal tract could be the second major source of enteric pathogens during the slaughtering process (33). Leakage from gastrointestinal tract could also cause widespread contamination. The preventive measures for reducing bacterial contamination during evisceration are tying the esophagus to prevent escape of ingesta, enclosing the bung to prevent escape of faeces and removal of the intact viscera. Studies indicated that inverted dressing i.e. dressing of the carcass head upwards can greatly reduce the rate of contamination (34). Cold-water carcass washes, although effective in removing macro-contamination are ineffective in removing microbial contamination. Trimming and washing can reduce gross contamination at heavily and moderately contaminated sites but they have no decontaminating effect on the carcass as a whole. This was observed in this study where there was no difference in the frequency of isolation of campylobacter between post-evisceration and after washing in sheep carcasses even though there was a substantial difference in the goat carcasses (Table 1).

Among the thermophilic campylobacters isolated from sheep and goat carcass, *C. jejuni* accounted for 72.5% and *C. coli* for 27.5% in the present study (Table 3). Similar findings have been reported that prevalence of *C. jejuni* in meats of animal origin (except in pork meat), dairy products and vegetables was higher than *C. coli* ranging from 45 to 89% (12, 20, 21). In this study, the antimicrobial susceptibility pattern of isolates was not performed because of laboratory facility and financial limitations. Otherwise, it would have given valuable information on the resistance pattern of the isolates. In conclusion, contamination of carcasses with *Campylobacter* spp. has indicated the need to apply good hygienic standards in the whole slaughtering process. In addition, tying the esophagus to prevent escape of ingesta, enclosing the bung to prevent escape of faeces and the intact removal of the viscera are some of the measures should be applied for reducing bacterial contamination during evisceration.

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References

1. Acha PN, Szyfres B. Zoonoses and communicable diseases common to man and animals. 3rd ed. PAHO Washington DC, 2001; 1: 67-78.
2. Ellen JB, Lance RP, Sydney MF. Epidemiology and pathogenesis of *Campylobacter* infection. In: Diagnostic Microbiology. 9th Ed MOSBY-Year Book Inc. Toronto, 1994: pp. 437-441.
3. Radostits OM. *Campylobacter* infection. In: Herd Health: Food Animal Production Medicine. 3rd ed. W.B. Saunders Company Philadelphia pp. 2001; 155-156.
4. Prescott JF, Munroe DL. *Campylobacter jejuni* enteritis in man and domestic animals. *J Am Vet Med Assoc* 1982; 181: 1524-1530.
5. Davis BD, Dulbecco R, Eisen HN, Gonsbery HS. *Campylobacter* species. In: *Microbiology. 4th Ed. The Murray printing Company* London 1990 pp. 586-87.
6. Lastovica AJ, Skirrow MB. Clinical significance of *Campylobacter* and related species other than *Campylobacter jejuni* and *C. coli* In: *Campylobacter*, Second Edition, Nachamkin I. & MJ. Blaser, eds. ASM Press, Washington DC, USA, 2000; 89-120.
7. Takahashi M, Koga M, Yokoyama K, Yuki N, Epidemiology of *Campylobacter jejuni* isolated from patients with Guillain–Barre and Fisher syndromes in Japan. *J Clin Microbiol* 2005; 43: 335–339.
8. Skirrow MB, Blaser MJ. Clinical aspects of *Campylobacter* infection. In: *Campylobacter*, Second Edition, Nachamkin I. & MJ. Blaser, Eds. 2000.
9. Aarestrup FM, Engberg J. Antimicrobial resistance of thermophilic *Campylobacter*. *Vet Res* 2001; 32:311-321.
10. Aarestrup FM. Association between the consumption of antimicrobial agents in animal husbandry and the occurrence of resistance bacteria among food animals. *Int J Antimicrob Agents* 1999;12: 279-285.
11. Gedlu E, Aseffa A. *Campylobacter* enteritis among children in northwest Ethiopia: A 1-year prospective study. *Ann Trop Paediatr* 1996; 16:207-212.
12. Asrat D, Hathaway A, Ekwall E. Studies on enteric campylobacteriosis in Tikur Anbessa and Ethio-Swedish children's Hospital, Addis Ababa, Ethiopia. *Ethiop Med J* 1999; 37:71-84.
13. Kassa T, Gebre-selassie S, Asrat D. The prevalence of thermotolerant *Campylobacter* species in food animals in Jimma Zone, southwest Ethiopia. *Ethiop J Health Dev* 2005; 9:225-229.
14. Dadi L and Asrat D. Prevalence and antimicrobial susceptibility profiles of thermotolerant *Campylobacter* strains in retail raw meat products in Ethiopia. *Ethiop J Health Dev* 2008;22:195-200.
15. Asrat D, Hathaway A, Sjogren E, Ekwall E, Kaijser B. The serotype distribution of *Campylobacter jejuni* and *Campylobacter coli* isolated from patients with diarrhea and controls at Tikur Anbassa Hospital, Addis Ababa, Ethiopia. *Epidemiol Infect* 1997; 118:91- 95.
16. Asrat D, Hathaway A, Ekwall E. Antimicrobial sensitivity pattern of *Campylobacter* strains isolated from patients in Tikur Anbassa and Ethio-Swedish Children's Hospitals, Addis Ababa, Ethiopia. *Ethiop J Health Dev* 1999; 13:41-44.
17. Beyene G, Haile-Amlak H. Antimicrobial sensitivity pattern of *Campylobacter* species among children in Jimma University Specialized Hospital, southwest Ethiopia. *Ethiop J Health Dev* 2004; 3:185-189.
18. Kassa T, Gebre-selassie S, Asrat D. Antimicrobial susceptibility patterns of thermo-tolerant C*ampylobacter* strains isolated from food animals in Ethiopia. *Vet. Microbiol* 2007; 119:82-87.
19. On SLW. Identification methods for *Campylobacters*, *Helicobacters*, and related organisms. *Clin Microbiol Rev* 1996; 9:405-422.
20. Friedman CR, Neimann J, Wagener HC, Tauxe RV. Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations. In: *Campylobacter*, Second Edition, Nachamkin I. & MJ. Blaser, eds. ASM Press, Washington DC, USA, 2000: 121-138.
21. Oberhelman RA, Taylor DN. *Campylobacter* infection in developing countries. *Campylobacter* In: Nachakin, Blaser, M.J. 2nd ed. Washington and I DC, ASM 2000: pp.139-153.
22. Whyte P, McGill K, Cowley D, et. al. Occurrence of *Campylobacter* in retail foods in Ireland. *Int J Food Microbiol* 2004; 95:111-118.
23. Hussain I, Mohammed SM, Akhtarb M, Khan A. Prevalence of *Campylobacter species* in meat, milk and other food commodities in Pakistan. Food *Microbiol* 2007; 24: 219-222.
24. Rosef O, Gondrosen B, Kapperud G , Underdal, B. Isolation and characterization of *Campylobacter jejuni* and *Campylobacter coli* from domestic and wild mammals in Norway. *Appl Environ Microbiol* 1983; 46: 855-859.
25. Vanderlinde PB, Shay B, Murray J. Microbiological status of Australian sheep meat. *J Food Prot* 1999: 62:380-385.
26. Cabrita J, Rodriguez J, Braganca F, Morgado C, Piers I, Goncalves AP. Prevalence, biotypes, plasmid profile and antimicrobial resistance of *Campylobacter* isolated from wild and domestic animals from north east Portugal. *J Appl Bacteriol* 1992: 73:279-285.
27. Fricker CR, Park RW. A two years study of the distribution of thermophilic *Campylobacter* in human, environmental and food samples from the reading area with particular reference to toxin production and heat-stable serotype. *J Appl Bacteriol* 1989; 66: 477-490.

28. Aquino MH, Pacheco AP, Ferreira MC, Tibana A. Frequency of isolation and identification of thermophilic Campylobacters from animals in Brazil. *Vet J* 2002; 164: 159-16.
29. Turkson PK, Lindquist KJ. Apperud K. Isolation of *Campylobacter* spp. and *Yersinia enterocolitica* from domestic animals and humans patients in Kenya. APMIS 1988; 96: 141-146.
30. Prescott JF, Bruin-Mosch CW. Carriage of *Campylobacter jejuni* in healthy and diarrheic animals. *Am J Vet Res* 1981; 42: 164-165.
31. Whyte P, McGill, Collins JD. An assessment of steam pasteurization and hot water immersion treatments for the microbiological decontamination of broiler carcasses. *Food Microbiology* 2003; 20:111-117.
32. Lasta JA, Pensel N, Masana M, Roderiguez R, Garcia PT. Microbial growth and biochemical changes on naturally contaminated chilled-beef subcutaneous adipose tissue stored aerobically. *Meat Science;* 39:149-158.
33. Byrne B, Dunne G, Lyng J, Bolton DJ. Microbiological carcass sampling methods to achieve compliance with 2001/471/EC and new hygiene regulations. *Res Microbiol* 2005; 156:104-6.
34. Bell RG, Hathaway SC. The hygienic efficiency of conventional and inverted lamb dressing systems. *J Appl Bacteriol* 1996; 81:225-34.