

Prevalence and Characteristics of *Campylobacter* Species Isolated from Gallbladder of Slaughtered Sheep in Van, (Eastern) Turkey

I. H. EKIN¹, K. GÜRTÜRK¹, A. ARSLAN², B. BOYNUKARA¹

¹Department of Microbiology, Faculty of Veterinary Medicine,

²Institute of Health Science, University of Yuzuncu Yil, Kampus-Van, Turkey

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Abstract

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To determine the prevalence of campylobacter species in gallbladder of sheep in Van, (Eastern) Turkey, a total of 220 gallbladder samples from healthy slaughtered sheep were examined bacteriologically in October 2000 and 2002. Of the 110 samples examined each year, 27 (24.6%) and 24 (21.8%) campylobacter strains were isolated, respectively. Of the 27 campylobacter strains isolated in the year 2000, 14 (51.9%) were identified as *C. jejuni*, 7 (25.9%) *C. fetus*, 3 (11.1%) *C. coli* and 3 (11.1%) *C. lari*. Similar results were obtained in the study performed in 2002, but *C. lari* could not be isolated. Growth and biochemical characteristics of all identified *Campylobacter* species with some exceptions were typical of each species. Six of 13 examined *C. fetus* strains grew well at both 25 °C and 42 °C in thioglycollate medium and on blood agar. *C. jejuni* strains differed from *C. coli* only by Na-hippurate hydrolysis test. Results of the present study revealed that *C. jejuni* is the most common campylobacter species isolated from gallbladders of sheep. The thermophilic campylobacters in significant proportions may cause contamination of carcass during slaughter and transmission of the food-borne pathogens to humans.

Prevalence, contamination, food-borne pathogens, Campylobacter species, bacteriology, identification, phenotype, strain, biochemistry

Campylobacters are important pathogens of humans and animals. *C. fetus* has been recognized as a cause of septic abortion, infectious infertility and diarrhoea in cattle and sheep (Varga et al. 1990; Gürtürk et al. 2000; Mannering et al. 2004). Thermophilic campylobacters such as *C. jejuni*, *C. coli* are also known as causal agents of abortions in sheep (Penner 1988; Gürtürk et al. 2002; Butzler 2004). *C. jejuni* and *C. coli* were found to be commensal in the rumens and small intestines of healthy calves and adult cattle. They cause acute gastroenteritis in human and enteritis in animals. *C. jejuni* is recognized as a causative agent of avian hepatitis of fowl (Butzler 2004; Tangvatcharin et al. 2005).

Nowadays, campylobacter species are a leading cause of food-borne diseases in the industrialized world. *C. jejuni* and *C. coli* are the most frequent species isolated from humans. The presence of campylobacter species in intestines and gallbladder of sheep may cause contamination of carcass during slaughtering and transmission of these food-borne pathogens to human beings by consumption of contaminated and low-heated sheep meat, meat products, liver or by direct contact with infected animals (Penner 1988; Butzler 2004; Blaser et al. 1983). Campylobacter species have been isolated from the intestines and gallbladders of apparently healthy sheep (Diker 1985; Wokatsch and Bockemühl 1988; Raji et al. 2000; Yazicioglu 2000). Previous studies (Diker 1985; Yazicioglu 2000; Ertas et al. 2003) indicated that the prevalence of *Campylobacter* spp. in the gallbladder of slaughtered sheep varied from 52.8% to 66% in some regions of Turkey.

Address for correspondence:

Dr. Ismail Hakki EKIN
Dept. of Microbiology, Faculty of Veterinary Medicine
University of Yuzuncu Yil
65080 Kampus-Van, Turkey

Phone: +90-432-2251128
Fax: +90-432-2251127
GSM: +90-532-4957220
E-mail: ihekin@yyu.edu.tr
<http://www.vfu.cz/acta-vet/actavet.htm>

The present study was performed to determine the prevalence of campylobacter species isolated from gallbladders of slaughtered sheep in Van, (Eastern)Turkey and to evaluate the biochemical characteristics for identifying campylobacter species.

Materials and Methods

Gallbladder samples

In October 2000 and 2002, 110 gallbladder samples for each year were collected from slaughtered sheep older than 1 year in Van, Turkey and were examined bacteriologically within 2 h of slaughter.

Isolation

A loop full of the gallbladder content was directly inoculated onto blood agar plates (Blood agar base No. 2, Oxoid-CM271, UK) containing 5% defibrinated sheep blood and Skirrow selective supplements (Oxoid-SR069E, UK). The selective agar plates were cultured at 37 °C for 48-72 h under aerobic and micro-aerobic atmosphere generated by using Campy-Gen (Oxoid-CN25, UK) in anaerobic jar (Oxoid-X1032, UK, without palladium catalyst) (Vandamme and Goossens 1992; On 1996).

Preliminary identification

Preliminary identification of campylobacter species was based on phenotypic characteristics; such as colony appearance, Gram staining, microscopic morphology, motility, catalase and oxidase reaction, nitrate reduction and fermentation of glucose (Holt et al. 1994; Nachamkin 1999).

Phenotypic identification

The following tests were performed for the identification of campylobacter species; H₂S production using lead acetate strips, hydrolysis of hippurate and indoxyl acetate, growth at 25 °C and 42 °C; growth in the presence of 1% glycine, 3.5% NaCl and 0.04% Triphenyl Tetrazolium Chloride (TTC); sensitivity to 30 µg nalidixic acid and cephalothine disks (Vandamme and Goossens 1992; Holt et al. 1994; On 1996). Growth and biochemical characteristics were examined in thioglycollate medium (Oxoid-CM391, UK). H₂S production was detected in brucella broth (Acumedia-7121, USA) with 0.02% cysteine by using lead acetate strips (Vandamme and Goossens 1992, Nachamkin 1999).

Results

A total of 27 (24.6%) and 24 (21.8%) campylobacter strains were isolated from gallbladders of slaughtered sheep in the years 2000 and 2002, respectively. All isolates were found to be Gram negative, motile, curved rods, catalase and oxidase positive, reducing nitrate, not utilizing glucose and growing microaerobically but not aerobically at 37 °C. Of the 27 strains isolated in the year 2000, 14 (51.9%) were identified as *C. jejuni*, 7 (25.9%) *C. fetus*, 3 (11.1%) *C. coli* and 3 (11.1%) *C. lari*. Similar results have obtained, but *C. lari* could not be isolated in the year 2002 (Table 1).

Table 1. Prevalence of *Campylobacter* species isolated from gallbladder of sheep at slaughterhouse in the years 2000 and 2002

Campylobacter species	Number (%) of the strains isolated in the years		Total
	2000*	2002*	
<i>C. fetus</i>	7 (6.4)	6 (5.5)	13 (5.9)
<i>C. jejuni</i>	14 (12.7)	14 (12.7)	28 (12.7)
<i>C. coli</i>	3 (2.7)	4 (3.6)	7 (3.2)
<i>C. lari</i>	3 (2.7)	-	3 (1.4)
Total	27 (24.6)	24 (21.8)	51 (23.2)

* A total of 110 samples were examined for each year

Further growth and biochemical characteristics of all examined campylobacter strains were typical of each species with some exceptions. The *C. coli* could be distinguished only by sodium-hippurate test from those of *C. jejuni*. Six *C. fetus* strains grew well at both 25 °C and 42 °C in thioglycollate medium and on blood agar. Except for *C. fetus* strains, 11 of 14 *C. jejuni* strains and all *C. coli* strains grew also in medium with presence of 0.04% TTC (Table 2).

Table 2. Some characteristics of 51 *Campylobacter* strains isolated from gallbladder of slaughtered sheep

Tests		<i>C. fetus</i>	<i>C. jejuni</i>	<i>C. coli</i>	<i>C. lari</i>
		(n: 13) Pos./ Neg.	(n: 28) Pos./ Neg.	(n: 7) Pos./ Neg.	(n: 3) Pos./ Neg.
H ₂ S	Lead acetate strip	13 / 0	28 / 0	7 / 0	3 / 0
Growth at	25 °C	13 / 0	0 / 28	0 / 7	0 / 3
	42 °C	6 / 7	28 / 0	7 / 0	3 / 0
Growth in presence of	1 % Glycine	13 / 0	28 / 0	7 / 0	3 / 0
	3.5 % NaCl	0 / 13	0 / 28	0 / 7	0 / 3
	0.04 % TTC*	0 / 6	11 / 3	4 / 0	ND
Sensitive to	Nalidixic acid	0 / 13	28 / 0	7 / 0	0 / 3
	Cephalothine	13 / 0	0 / 28	0 / 7	0 / 3
Hydrolysis of	Na-Hippurate	0 / 13	28 / 0	0 / 7	0 / 3
	Indoxyl acetate	0 / 13	28 / 0	7 / 0	0 / 3

* The test was performed for the strains isolated in the year 2002

ND: Not Detected

Discussion

In the present study, the isolation rate of campylobacter strains from gallbladders of slaughtered sheep was determined as 24.6 - 21.8% in the years 2000 and 2002, respectively. These findings appeared to be lower than other reports (Diker 1985; Yazicioglu 2000; Ertas et al. 2003) in Turkey. In these reports, the prevalence of *Campylobacter* spp. in the gallbladders of slaughtered sheep was found to be 57.3%, 52.8% and 66%, respectively. It has been reported that the isolation rate of campylobacter species from intestines or gallbladder of lambs and calves are higher than those of sheep and cattle (Diker 1985; Giacoboni et al. 1993). The lower isolation rate obtained in this study might be due to the fact that sheep examined in this study were older than one year of age.

Previous reports (Clarck and Mounbough 1979; Yazicioglu 2000) indicated that *C. fetus* is more prevalent than *C. jejuni* in the gallbladders of slaughtered sheep. In contrast, the results of this study revealed that the *C. jejuni* was found to be predominant campylobacter species in the gallbladder of slaughtered sheep. The distribution of campylobacter species were 12.7% *C. jejuni*, 6.4% *C. fetus* and 2.7% *C. coli* and *C. lari* in the year 2000. Similar results were also obtained in 2002. The isolation rate of *C. jejuni*, *C. fetus* and *C. coli* were 12.7%, 5.5% and 3.6%, respectively, but *C. lari* could not be isolated in 2002. The differences in the occurrence and distribution of campylobacter species in gallbladder of sheep might be due to the differences in environmental and breeding conditions between areas or countries.

In agreement with other reports (Diker 1985; Yazicioglu 2000; Raji et al. 2000) *C. coli* was also detected in gallbladders of sheep examined in this study. *C. coli* are found to be common in humans and chickens but rare in sheep and cattle (Wokatsch and Bockemühl 1988; Bae et al. 2005). On the other hand, *C. jejuni* and *C. coli* could be recognized as separate species based on the hippurate hydrolysis reaction (Griffiths and Park 1990; Waino et al. 2003). The speciation was also confirmed using DNA-DNA hybridization techniques (Vandamme and Goossens 1992). Although the hippurate hydrolysis is a useful test for distinguishing *C. coli* and *C. jejuni* strains, there are some misidentifications with wild hippurate-negative *C. jejuni* strains (Griffiths and Park 1990; Waino et al. 2003). Totten et al. (1987) reported that the hippurate-negative campylobacter strains were identified as *C. jejuni* (20%), *C. coli* (78%) and *C. lari* (2%) by DNA hybridization test. Ronner et al. (2004) indicated that the *C. jejuni* strains, 5% from human and 10% from chicken, found to be negative with Na-hippurate test after genotyping

based on PCR/REA. In the present study, both thermophilic campylobacter strains differed only by hippurate test.

In addition, our study indicated that the tests for growth in presence of TTC and hydrolysis of indoxyl acetate appeared to be more characteristic of *C. jejuni* (except to three strains) and *C. coli* strains, but not for *C. fetus* and *C. lari* which were negative by both tests (On 1996; Holt et al. 1994; Nachamkin 1999).

However, six strains of *C. fetus* grew well at both 25 °C and 42 °C, and this property did not change after several culture passages. Because all other characteristics were typical, these thermophilic strains were identified as *C. fetus* (Penner 1988; Holt 1994). The thermophilic strains of *C. fetus* isolated from humans (Anstead et al. 2001; Woo et al. 2002) and from aborting sheep (Varga et al. 1990) have already been reported.

The *C. lari* strains identified in this study were resistant to both nalidixic acid and cephalothine, negative in hippurate test and grew well at 42 °C but not at 25 °C as reported by VanDamme and Goossens (1992) and Raji et al. (2000). These strains were described as "NARTC" (nalidixic acid resistant thermophilic campylobacter) strains. *C. lari* is rarely isolated from the intestines of healthy sheep or cattle and did not appear to be associated with disease (Penner 1988; Butzler 2004; Diker 1985).

In conclusion, the prevalence of campylobacter species in gallbladder of slaughterhouse sheep was found to vary from 21.8% to 24.6% in Van, (Eastern) Turkey. *C. jejuni* are found to be the most common campylobacter species in gallbladder of sheep. Presented data showed also that the gallbladder of sheep containing thermophilic campylobacters in significant proportions may cause contamination of carcass during slaughter and transmission of the food-borne pathogens to humans. Even if the biochemical characteristics are suitable to confirm the preliminary identification of the campylobacter isolates on the species level, additional molecular biological techniques should be applied to reliable identification of the hippurate-negative campylobacter isolates.

Rozšíření a charakteristika druhů bakterie Campylobacter izolovaných ze žlučníku jatečných ovcí v provincii Van ve východním Turecku

Pro určení rozšíření druhů bakterie rodu Campylobacter ve žlučníku ovcí v provincii Van ve východním Turecku bylo bakteriologicky vyšetřeno celkem 220 vzorků žlučníku zdravých jatečných ovcí v říjnu roku 2000 a 2002. Ze 110 vzorků vyšetřených v každém roce bylo izolováno 27 (24,6 %), resp. 24 (21,8 %) druhů bakterie Campylobacter. Z 27 druhů izolovaných v roce 2000 bylo 14 (51,9 %) identifikováno jako *C. jejuni*, 7 (25,9 %) jako *C. fetus*, 3 (11,1 %) jako *C. coli* a 3 (11,1 %) jako *C. lari*. Podobných výsledků bylo dosaženo při výzkumu provedeném v roce 2002, avšak *C. lari* se izolovat nepodařilo. Typický pro všechny identifikované druhy bakterie Campylobacter byl, až na několik výjimek, růst a biochemická charakteristika. Šest ze 13 zkoumaných kmenů *C. fetus* dobře rostlo jak při 25 °C, tak při 42 °C v thioglykolátovém médiu a na krevním agaru. Kmen *C. jejuni* se odlišoval od *C. coli* pouze při hydrolyze Na-hippurátu. Výsledky této studie ukázaly, že *C. jejuni* je nejběžnějším druhem bakterie Campylobacter izolovaným ze žlučníku ovcí. Termofilní bakterie rodu Campylobacter se mohou významně podílet na kontaminaci masa při porážce a přenosu patogenů potravinami na lidi.

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