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ISOLATION OF CAMPYLOBACTER JEJUNI, CAMPYLOBACTER COLI AND CAMPYLOBACTER LARIDIS FROM INTESTINE OF BROILERS

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Broiler barsaklarından Campylobacter jejuni, Campylobacter coli ve Campylobacter laridis izolasyonu

Özet: *Termofilik kampilobakterlerin izolasyonu amacıyla* 150 *adet tavuk barsağı incelendi. Ileum mukozasından ekim yapılan Preston selektif besiyerleri,* 42 °*C de mikroaerofilik atmosferde* 48 *saat inkübe edildi. Morfolojik olarak Campylobacter şeklinde ayrılan suşların identifikasyonları, üreme, tolerans ve biyokimyasal testler ile yapıldı. C. laridis nalidiksik aside dirençli olması, C. jejuni pozitif hippurat testi, C. coli negatif hippurat testi ile ayrıldı.*

İncelenen toplam 150 *adet örneğin tümünden termofilik kampilobakterler izole edildi. Bu suşlardan* % 42.7 *si C. jejuni,* % 51.3 *ü C. coli ve* % 2.7 *si C. laridis olarak identifiye edildiler. Bu türlerin izolasyonu, Türkiye'de tavuklardan ilk kez bildirilmektedir.*

Summary: *In this study, intestines of* 150 *poultry were examined to isolate the thermophilic campylobacters. Samples taken from ileum were cultured on Preston selective medium and incubated at* 42 °*C in microaerophilic atmosphere for* 48 *hours. The further differentiation of strains identified as Campylobacter sp. was done by growth, tolerance and biochemical tests.*

All samples investigated were found positive for campylobacters. The distribution of C. jejuni, C. coli and C. laridis among these isolates was 42.7, 51.3 *and* 2.7 *per cent, respectively. This report presents the first isolation of these three species from poultry in Turkey.*

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Introduction

Bacteria belonging to the *genus Campylobacter* have attracted considerable interest during the past decade due to an increasing frequency of isolations from animal and human sources. The most widely isolated species of this genus is *Campylobacter jejuni*. Other two species of this group called "thermophilic campylobacters" which show minor phenotype differences from each other are *Campylobacter coli* and *Campylobacter laridis*. Thermophilic campylobacters have been isolated from intestinal tracts of wild and domestic mammals and birds (6, 9, 17), and considered as a part of normal intestinal flora of poultry (17). Recent studies showed that the prevalence of these organisms in the feces of birds ranged from 40 to 100 per cent (20, 30). The microorganism spreads through the flock from one or more sources and continues to survive in a high percentage of poultry, both at the brooder facility and at the processing plant. Recent studies have confirmed that campylobacter can readily colonize the gastrointestinal tract of young chicks (14). In the past, it had also been suggested that bacteria, possibly similar to *C. jejuni*, have been implicated in infectious hepatitis of chickens (1).

Studies throughout the world have implicated *C. jejuni* as the causative agent of acute gastroenteritis in men in 3 to 14 per cent of cases requiring medical attention (3, 18). Several investigators have reported that poultry products contaminated with *C. jejuni* are an important source of transmission (12, 15). Recent studies have revealed isolation rates of *C. jejuni* from all parts of processed chickens that reached to 83 per cent (26). Campylobacters have also been detected in fresh and frozen chicken at market level, and they are able to survive for extended periods in frozen or refrigerated meat (10, 13).

The present study was undertaken to assess the occurrence of thermophilic campylobacters in the intestines of broiler chickens.

Materials and Methods

Animals: A total of 150 chickens from 2 poultry flocks were investigated. Ranch A was a broiler brooder flock having 2000 animals, aged 45 weeks. Ranch B had 5000 animals, aged 8 weeks. Whole intestinal tracts of 100 chickens from ranch A and 50 chickens from

ranch B were collected during slaughtering and transported to the laboratory in ice box within 4 hours.

Media: Campylobacter blood agar supplemented with Preston antibiotic combination (4) was used for the isolation of thermophilic campylobacters. This medium contained following ingredients per liter of distilled water: blood agar base No. 2 (Oxoid), 40 g; polymyxin B, 5000 IU; rifampicin, 10 mg; trimethoprim lactate, 10 mg; actidione, 100 mg and 7 % defibrinated sheep blood. Biochemical and growth tests were performed in nutrient broth No. 2 (Oxoid) containing 0.16 % agar and on Mueller-Hinton agar (Oxoid). The isolates were subcultured in Thiol Medium (Difco) and preserved in Brucella broth (Difco) at – 70 °C.

Intestinal sampling and isolation: When the intestines were sampled, the external surface of ileum was seared with a hot spatula prior to incision of ileum wall with a sterile scalpel. Then a steril swab introduced into the intestinal lumen to collect sample. Swabs contaminated with intestinal content were seeded on Campylobacter selective agar plates. Plates were incubated in anaerobic dessicators without catalyst and microaerophilic condition was obtained by using Gas Generating Kit (Oxoid). Dessicator was held at 42 °C for 48 hours. After incubation, any growth on plates observed and colonies similar to the typical morphology of campylobacters were selected for further examination.

Characterization: Suspected colonies were stained with Gram staining method and investigated for motility under dark field illumunation. Biochemical tests (oxidase, catalase, nitrate reduction, selenite reduction), tolerance tests (3.5 % sodium chloride, 1 % glycine, 30 mcg/ml cephalothin) and growth tests (25 °C, 30.5 °C, 45.5 °C) were used for the identification of thermophilic campylobacters. Thermophilic campylobacters were further divided into three species by hippurate hydrolysis test and nalidixic acid (30 mcg/ml) susceptibility test.

The biochemical, tolerance and growth tests used for the differentiation of thermophilic campylobacters from each other and other campylobacters are shown in Table 1.

Table 1. Phenotype characteristics of Campylobacter species[a]

	C. jejuni	C. coli	C. laridis	C. fetus	C. sputorum	Others[x]
Oxidase	+	+	+	+	+	+
Catalase	+	+	+	+	+	±
Nitrate	+	+	+	+	±	±
H_2S	+	+	+	d	+	±
Hippurate	+	+	−	−	−	−
25 °C	−	−	−	±	−	−
42 °C	+	±	−	−	d	±
% 1 Glycine	+	±	+	d	d	±
% 3.5 NaCl	+	−	−	−	d	±
Nalidixic acid[b]	S	S	R	R	d	RS
Cephalothin[b]	R	R	R	S	S	S

a symbols: −, 90 % or more of the strains are positive; +, 90 % or more of the strains are negative; d, 11 to 98 % of the strains are positive; ±, some species are positive; R, resistant; S, sensitive; RS, some species are resistant.

* Others: C. fecalis, C. hyointestinalis, C. concisus, C. cinaedi, C. fenneliae, C. pyloridis.

Results

Thermophilic campylobacters were isolated from all (100 %) of 150 intestinal samples of broiler chickens. Of 150 isolates identified, 51.3 % were *C. coli*, 42.7 % were *C. jejuni* and 2.7 % were *C. laridis* (Table 2). Five strains identified as Campylobater sp could not survive while subculturing before further identification. Since they grew

Table 2. Isolation of thermophilic campylobacters from poultry

Species	Intestinal samples		
	Ranch A (n = 100)	Ranch B (n = 59)	Total (n = 150)
C. jejuni	43 (43.0%)	21 (42.0%)	64 (42.7%)
C. coli	52 (52.0%)	25 (50.0%)	77 (51.3%)
C. laridis	3 (3.0%)	1 (2.0%)	4 (2.7%)
Unidentified	2 (2.0%)	3 (6.0%)	5 (3.3%)

at 42 °C, they were thermophilic campylobacters. The prevalence of *C. jejuni*, *C. coli* and *C. laridis* were found similar in each flock. Isolated strains showed typical characteristics of thermophilic campylobacters. Phenotype charecteristics of thermophilic campylobacters isolated are shown in Table 3. All strains of *C. jejuni* hydrolysed hippurate, but *C. coli* did not. All *C. laridis* strains were resistant to nalidixic acid but other two strains were not.

Table 3. Phenotype characteristics of C. jejuni, C. coli and C. laridis isolated from poultry (%)[a]

Tests	C. jejuni (n – 64)	C. coli (n = 77)	C. laridis (n = 4)
Curved rods	100.0	100.0	100.0
Oxidase	100.0	100.0	100.0
Catalase	100.0	100.0	100.0
Nitrate reduction	100.0	100.0	100.0
H_2S production	100.0	100.0	100.0
Hippurat hydrolysis	100.0	0.0	0.0
25 °C	0.0	0.0	0.0
Growth at 30.5 °C	7.8	70.1	75.0
45.5 °C	53.1	36.3	25.0
Tolerans to 1 % Glycine	100.0	97.4	100.0
Nalidixic acid[c]	0.0[b]	0.0	100.0
Cephalothin[c]	100.0	100.0	100.0

a = Percentage of positive result, b Percentage of resistant strains, c, 30 mcg/ml.

Discussion and Conclusion

In this study, thermophilic campylobacters were isolated from 100 per cent of animals. This isolation rate is one of the highest in the world. In a previous study, prevalence of *C. jejuni* in ceca of broilers was also 100 per cent (30).

C. jejuni has been frequently isolated from the intestinal tract and feces of healthy poultry throughout the world. This microorganism has been recovered from 74 % of rectal swabs from broilers in Australia (23), 10 % of Norwegian poultry (21), 72 % from broilers in Sweden (14) and 41 % from broilers in Israel (20). In the present study the incidence of *C. coli*, *C. jejuni* and *C. laridis* was 51.3, 42.7 and 2.7 per cent, respectively. The present results indicate that thermophilic campylobacters, particularly *C. coli* and *C. jejuni*, are also widespread among poultry population in Turkey.

It is known that there are several species of Campylobacter and most attention has been focused on *C. jejuni* due to its contribution to many infections. Infections due to *C. coli* which is closely related to *C. jejuni*, appear to share many clinical and epidemiological characteristics. In this study, the prevalence of *C. coli* was very high when comparing with the results of other surveys. Only a few report indicate the presence of *C. coli* and *C. laridis* in domestic poultry (12, 20). The cause of this situation is most likely the old classification of campylobacters in these previous studies. Before new classification of ther-

mophilic campylobacters, *C. coli* strains had been considered as the hippurate negative biotype of *C. jejuni* (11), and *C. laridis* had been named as nalidixic acid resistant thermophilic campylobacter (NARTC). Although the strains similar to *C. coli* and *C. laridis* were isolated in the most of previous works, all these strains were named as *C. jejuni*. Recently, hippurate negative strains of *C. jejuni* were transferred to *C. coli* and NARTC strains to *C. laridis* (2, 15). As a result of new nomenclator, recent reports indicating the presence of *C. coli* and *C. laridis* have been seen in literature (12, 20). Likewise, *C. jejuni* had been previously isolated from cattle and sheep, (5, 6) and from human with gastroenteritis (19, 29) in Turkey. Since they had been conducted before new classification, many strains of these studies were named as *C. jejuni*, instead of *C. coli* and *C. laridis*. In any case *C. coli* predominated in this study while *C. jejuni* predominated in other studies from all over the world.

The source of campylobacter contamination and the cause of high incidence of campylobacters have been investigated by several workers. Montrose et al. (16), infected the SPF chickens with Campylobacter by using experimentally contaminated litter and showed that litter was an important source of campylobacters. In the present study, high isolation rate may be related to litter contamination. Shane et al. (22) demonstrated in a series of experiments that flies could transmit the *C. jejuni* to SPF chickens under controlled laboratory conditions. Since the present study was conducted in winter season during which flies do not appear, this source could not be the cause of transmission of campylobacters to broilers.

Studies on the incidence of Campylobacter species in broiler flocks monitored from hatching to slaughter have shown that these microorganisms colonize the intestinal tracts of birds at the age of 4–8 weeks (17, 27, 28). In the previous studies, the lack of isolation of campylobacters from eggs of hens which are fecal excreter of this agent (8) and the lack of attempt to transmit infection vertically (23) support these findings. In the present study,animals were 8 and 45 weeks of age and these ages are the most susceptible period for the colonization of campylobacters in the intestine of poultry.

Another possible reason of high prevalence of campylobacters in this study was the collection of specimens from mucosal surface of intestine. When comparing the isolation studies in which both mucosal

swabs and faeces were used as inoculum, it has been determined that isolation rate from mucosal specimens was always higher than those from faeces (7). This can be attributed the affinity of microorganism to mucosal surface, particularly to mucus layer.

The most important aspect of high prevalence of campylobacters in poultry is the possibility of transmission to human. The intestinal content of broilers always contaminates the processing plant during slaughtering. Kinde et al. (13) reported that *C. jejuni* was found in 83 % of chicken wings analysed on the day of arrival at supermarkets. Other studies on the same subject in which various body parts of broiler carcasses were examined for campylobacters at slaughterhouse or at the supermarket level have showed that isolation rates from carcasses were as high as intestines (12, 26, 30). Many food-borne outbreaks of campylobacter enteritis in men due to undercooked broiler have been reported (3, 10, 18). Shanker et al. (24) found that most of chicken and human isolates from same area were of identical biotypes. In Turkey, limited numbers of paper indicates Campylobacter infection in human (19, 29). Most likely, thermophilic campylobacters from poultry cause enteric infection in human in Turkey.

Further studies on the distribution of campylobacters among poultry and men should be performed and the epidemiological aspects of campylobacter infections should be determined. On the other hand, despite the fact that campylobacters can survive in the intestine of poultry as normal flora such a high prevalence, it is necessary to understand in which conditions this agent causes the disease called bacterial infectious hepatitis or which mechanism protect the animals from this disesase.

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