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Research Article

The Prevalence of *Campylobacter jejuni* in Various Sources in Kayseri, Turkey, and Molecular Analysis of Isolated Strains by PCR-RFLP*

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Abstract: The objective of this study was to isolate, identify, and genotype *Campylobacter jejuni* from various sources in the province of Kayseri, Turkey. A total of 6667 samples consisting of 5167 human fecal swabs, 600 dog rectal swabs, 600 cattle gallbladders, and 300 chicken carcasses were examined. The samples were plated onto mCCDA (cefoperazone charcoal desoxycholate agar) agar. In order to identify *C. jejuni*, phenotypic tests and PCR (polymerase chain reaction) were performed. *C. jejuni* was isolated in 1.43%, 43.50%, 31.16%, and 56% of the human, dog, cattle, and chicken samples, respectively. Among the 690 *C. jejuni* strains that were isolated during the study period, 200 *C. jejuni* strains (50 strains from each species) were randomly selected. The selected strains were typed by using PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) fla-typing. *Dde*I and *Hin*fI restriction enzymes were used for molecular typing. Following the *Dde*I enzyme application, the strains produced various numbers of bands between 4 and 7, with a total of 20 different band profiles. No similar band profiles were seen among the strains isolated from different sources. It was found that *Hin*fI was not a more discriminative enzyme for fla-typing of *C. jejuni* isolates.

Key Words: Human, dog, chicken, cattle, *Campylobacter jejuni*, PCR-RFLP (fla-typing)

Kayseri'de (Türkiye) Çeşitli Kaynaklarda *Campylobacter jejuni*'nin Prevalansı ve İzole Edilen Suşların PCR-RFLP ile Moleküler Analizi

Özet: Bu çalışma Kayseri yöresinde çeşitli kaynaklardan *Campylobacter jejuni*'nin izolasyonu, identifikasyonu ve identifiye edilen suşların genetik olarak tiplendirilmesi amacıyla yapıldı. Çalışma kapsamında, 5167 insan dışkı svabı, 600 adet köpek rektal svabı, 600 adet sığır safra kesesi ve 300 adet (paket) tavuk eti olmak üzere toplam 6667 adet numune incelendi. Örnekler izolasyon amacıyla mCCDA (cefoperazone charcoal desoxycholate agar)'a ekildi. *C. jejuni*'nın identifikasyonunda fenotipik testler ve PCR (Polymerase Chain Reaction)'den yararlanıldı. İnsan, köpek, sığır ve tavuk örneklerinden sırasıyla % 1,43, % 43,50, % 31,16 ve % 56 oranlarında *C. jejuni* izole edildi. Çalışma periyodu boyunca izole edilen 690 adet *C. jejuni* suşu içerisinden 200 adet seçilerek PCR-RFLP, (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) fla-typing yöntemi ile tiplendirildi. Moleküler tiplendirme amacı ile *Dde*I ve *Hin*fI restriksiyon enzimleri kullanıldı. *Dde*I enzimi ile muameleden sonra suşlar 4 ile 7 arasında band oluşturdu ve incelenen tüm suşlar 20 farklı band profili gösterdi. Farklı kaynaklardan izole edilen suşlar arasında benzer band profili veren suşlara rastlanmadı. Bu çalışmada *Hin*fI enziminin suşların tiplendirilmesinde ayırıcı olmadığı belirlendi.

Anahtar Sözcükler: İnsan, köpek, tavuk, sığır, *Campylobacter jejuni*, PCR-RFLP (fla-typing)

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Introduction

Campylobacter is commonly found in the gastrointestinal tracts of domestic and wild animals and is commensal (1,2). Many strains, however, particularly *Campylobacter jejuni* (*C. jejuni*), are enteric human pathogens. It is widely assumed that campylobacteriosis is primarily a food-borne disease. Contaminated meat, milk, and water are thought to be the major sources of human infection. Domestic pets, wild birds, and wild animals are also potential sources of *C. jejuni* infection in humans. Transmission occurs through the consumption of contaminated water and animal products (e.g., meat and milk), direct contact with infected animals, or handling undercooked poultry (3).

Several strain typing methods (e.g., phenotyping and genotyping) have been developed to understand the epidemiology of and to identify the transmission routes of *C. jejuni,* particularly in regard to humans. Although various phenotyping methods have been described, such as serotyping, biotyping, and phage-typing, these methods require specialist skills and a reagent, and are time consuming. It is also difficult to standardize these methods globally (4). Recently, several new genotyping techniques have been developed, including ribotyping, pulsed-field gel electrophoresis (PFGE), polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), flagellin typing (fla-typing), random amplified polymorphic DNA (RAPD), and amplified fragment length polymorphism (AFLP) (4,5).

The flagellin gene locus of *C. jejuni* contains 2 flagellin genes (*fla*A and *fla*B). This locus is suitable for PCR-RFLP analysis of PCR products because both genes are highly conserved, and variable regions are present. Thus, it has been reported that the use of a primer specifically designed for the amplification of fla in PCR-RFLP (fla-typing) is a useful, reliable, simple, and valuable subtyping technique for epidemiological studies (4).

The current study was undertaken to determine the prevalence of *C. jejuni* in various sources in Kayseri, Turkey. A secondary objective was the detection of *C. jejuni* subtypes using PCR-RFLP (fla-typing).

Materials and Methods

Bacterial strains

Between September 2002 and August 2003, 619 *C. jejuni* strains were recovered from different sources. The origin and number of these isolates are presented in Table 1. Human samples were taken from diarrheic patients. The dog and cattle samples were obtained from healthy animals. *C. jejuni* NCTC 11168 was used as the reference strain.

Isolation of enteric campylobacters

Modified CCDA (mCCDA) (LAB M lab 112) and a selective supplement (LAB M, cefoperazone-amphotericin, X112) were used for primary isolation of enteric campylobacters. Incubation was performed under micro-aerobic conditions for 24 to 48 h at 37 °C. All strains were identified using classical methods (2,6).

Identification of *C. jejuni*

A. Phenotyping assay

C. jejuni was identified by observing characteristic morphology and motility using phase contrast microscopy and also by using a phenotyping assay, which included growth patterns at various temperatures (25 °C and 42 °C), catalase production, oxidase reaction, hippurate hydrolysis, H_2S production, and susceptibility to nalidixic acid and cephalothin (2,7).

B. Identification of *C. jejuni* by PCR

C*euE* gene-specific primers (JEJ1 5'-CCT GCT ACG GTG AAA GTT TTG C-3' and JEJ2 5'-GAT CTT TTT GTT TTG TGC TGC-3') were used for identification of *C. jejuni* (8).

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP), (fla-typing)

Among the 690 *C. jejuni* strains that were isolated during a 1-year period, 200 *C. jejuni* strains (50 human, 50 chicken, 50 cattle, and 50 dog) were randomly selected. They were genotyped by a slightly modified PCR-RFLP fla-typing method, which is described elsewhere (9).

Bacterial DNA was prepared using a commercial DNA isolation kit (Genomic DNA Purification Kit, Fermentas, Lithuania). DNA concentrations were measured using a spectrophotometer (A_{260}) and diluted with sterile water to approximately 20 ng/µl.

*Fla*A genes (approximately 1700 bp) were amplified with specific primers (A1: 5'-GGA TTT CGT ATT AAC ACA AAT GGT GC-3', A2: 5'-CTG TAG TAA TCT TAA AAC ATT TTG-3') and digested separately using *Dde*I (Promega, USA) and *Hin*fI (Fermentas, Lithuania). Amplified and digested fragments were visualized using a GeneSnap-Gene Genius Bio Imaging System (Syngene, Cambridge, UK) and analyzed using Gene Tools software from Syngene. Genetic similarity among strains was calculated on a simple matching coefficient (10). The size of digested fragments on the gel was calculated from migration distances using UPGMA (unweighted pair group method with arithmetic mean) algorithms (11).

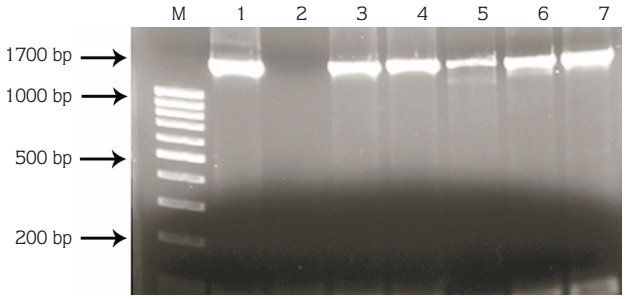

Figure 1. PCR amplification product of the *C. jejuni fla*A gene (approximately 1700 bp). M: 100 bp DNA ladder, Gene Ruler™; 1: Positive control; 2: Negative control; 3: Human *C. jejuni* isolate; 4: Dog *C. jejuni* isolate; 5: Cattle *C. jejuni* isolate; 6 and 7: Chicken *C. jejuni* isolate.

Results

Isolation of enteric campylobacters and identification of *C. jejuni*

The number of enteric campylobacters and the isolation rates of *C. jejuni* are given in Table 1. All presumptive *C. jejuni* strains identified with phenotyping methods were found to be positive by PCR; 793 bp fragments were observed on agarose gel.

PCR-RFLP fla-typing

PCR products amplified with the *fla*A gene-specific primer were present in bands of 1700 bp (Figure 1). After the digestion of the amplicon with *Dde*I and *Hin*fI, bands ranging from 100 to 1100 bp were detected.

*Dde*I restriction

Analysis of the 200 strains selected randomly from different isolates resulted in 20 different band profiles consisting of 4 to 7 bands each, after digestion with *Dde*I. The patterns of each band were evaluated as a group. No relationship among the strains of different origins could be detected (Table 2).

Fifty human isolates formed 4 different groups; 28 isolates were detected in the first group, 8 in the second group, and 7 isolates each in the third and fourth groups (Figure 2). Similarity levels among the groups were 57.14% to 66.67%.

Table 1. Number of enteric campylobacters and isolation rates of *C. jejuni* recovered from different sources.

	No. of samples	No. of enteric campylobacters	*C. jejuni*	
			No. of positive samples	Isolation rate (%)
Humans *	5167	108	74	1.43
Dogs**	600	331	261	43.50
Cattle**	600	272	187	31.16
Chickens	300	230	168	56
TOTAL	6667	941	690	10.34

* Fecal samples were taken from diarrheic patients

** Dog rectal swab samples and cattle gallbladders were taken from healthy animals

Table 2. Number of band patterns of *C. jejuni* isolates after *Dde*I enzyme digestion.

Source of isolates	Numbers of isolates	Band patterns	Similarity level (%)
Humans	28	D1	57.14-66.67
	8	D2	
	7	D3	
	7	D4	
Dogs	7	D5	52.63-70
	9	D6	
	10	D7	
	11	D8	
	13	D9	
Cattle	5	D10	47.06-75
	9	D11	
	10	D12	
	11	D13	
	15	D14	
Chickens	5	D15	50-77.78
	6	D16	
	7	D17	
	8	D18	
	11	D19	
	13	D20	
Total	200	20	

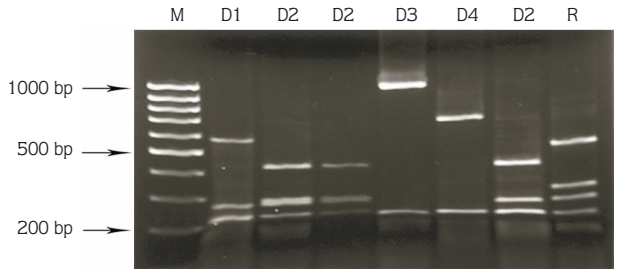

Figure 2. RFLP band patterns generated by *Dde*I digestion of the PCR product from *fla*A of human *C. jejuni* isolates. M: 100 bp DNA ladder; R: Reference strain (*C. jejuni* NCTC 11168).

Fifty dog isolates formed 5 different groups; 7 isolates were detected in the first group, 9 in the second, 10 in the third, 11 in the fourth, and 13 in the fifth (Figure 3). Similarity levels among the groups were 52.63% to 70%.

Fifty cattle isolates formed 5 distinct groups; 5 isolates were detected in the first group, 9 in the second, 10 in the third, 11 in the fourth, and 15 in the fifth (Figure 3). Similarity levels among the groups were 47.06% to 75%.

Fifty poultry isolates formed 6 different groups; 5 isolates were detected in the first group, 6 in the second, 7 in the third, 8 in the fourth, 11 in the fifth, and 13 in the sixth (Figure 3). Similarity levels among the groups were 50% to 77.78%.

*Hin*fI restriction

Analysis of the 200 strains resulted in 2 different banding patterns formed by 2 to 3 bands (Table 3). In all, 190 isolated strains of different origins were detected in the first group and 10 strains were detected in the second group (Figure 4). In the first group, 40 human,

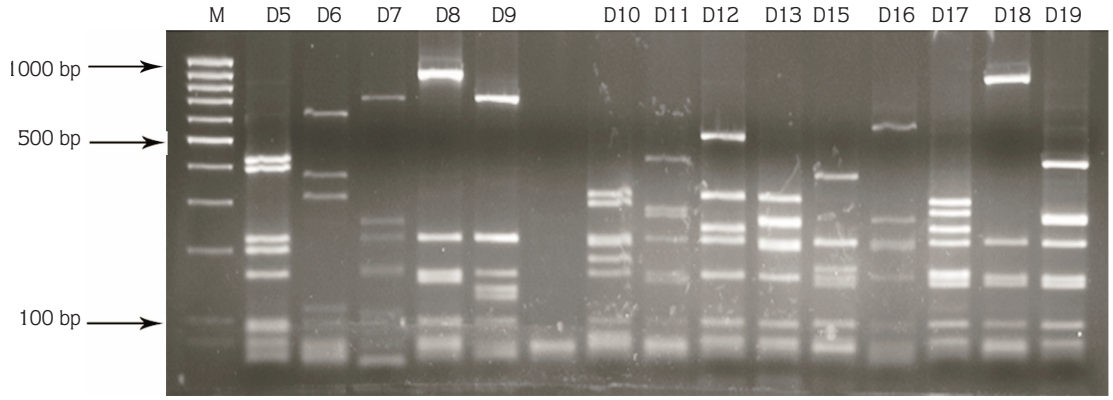

Figure 3. RFLP band patterns generated by *Dde*I digestion of the PCR product from *fla*A of *C. jejuni* isolated from dogs, cattle, and chickens. M: 100 bp DNA ladder; D5-D9: Dog isolates: D10-D13: Cattle isolates; D15-19: Chicken isolates.
* Patterns D14 and D20, isolates obtained from cattle and chickens, respectively, are not represented in this figure.

Table 3. Number of band patterns of *C. jejuni* isolates after *Hin*fI enzyme digestion.

Source of isolates	Numbers of isolates	Band patterns	Similarity level (%)
Humans	40	H1	57.14
	10	H2	
Dogs	50	H1	
Cattle	50	H1	
Chickens	50	H1	
Total	200	2	

and all dog, cattle, and chicken strains were detected. The rest of the human strains were detected in the second group. The similarity level between the 2 groups was 57.14%.

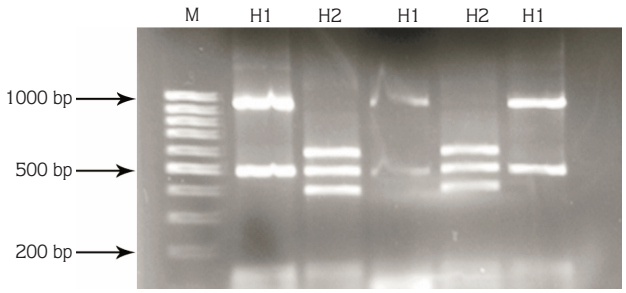

Figure 4. RFLP patterns generated by *Hin*fI digestion of the PCR product from *fla*A of *C. jejuni* M: 100 bp DNA ladder.

Discussion

Various studies have revealed that campylobacters present in the intestinal contents of chickens spread to their carcasses during slaughter, contaminating the carcasses, thus resulting in a public health risk (12). Isolation rates of *C. jejuni* from contaminated carcasses show variability. The *C. jejuni* rates found in chicken carcasses in different countries included 56% by Yıldız and Diker (13), 54% by Kwiatek et al. (14), 61% by Shih (15), 41% by Quinones-Ramirez et al. (16), and 50% by Özer and Ergün (17).

C. jejuni is commensally present in the intestinal flora of cattle (18,19) and dogs (20,21). In addition to the above-mentioned chicken carcasses, dogs and cattle also present a risk factor for human campylobacteriosis. Torre and Tello (20) isolated *C. jejuni* from healthy dogs at a

rate of 14.36% and Sandberg et al. (21) isolated it at a rate of 3%. Diker and İstanbulluoğlu (22) found *C. jejuni* in 62% of healthy cattle and 27% of healthy calves. Diker (6) found *C. jejuni* at the rate of 14% in both cattle gallbladders and stool samples. Çetin et al. (23) found it in 7% of cattle feces.

In the present investigation, *C. jejuni* was isolated at a rate of 56% from chicken carcasses, 31.16% from cattle gallbladders, and 43.50% from dog rectal swabs. The *C. jejuni* rate determined in this study was similar to those found in previous studies of chicken intestines in Turkey and other countries; however, the isolation rate of *C. jejuni* in dogs and cattle was higher than those found in other studies. Differences in isolation rates of *C. jejuni* may be attributed to several factors, such as sample size, medium, and isolation and identification procedure.

Compared to other pathogens, campylobacters are the most frequently isolated agents causing gastroenteritis in developed countries (3). In Turkey, *C. jejuni* isolation rates in humans with enteritis were found to be 7.5% by Işık et al. (24), 8.80% by Yıldırım et al. (25), and 2.25% by Aktaş and Tuncel (26).

In our study, *C. jejuni* was isolated at the rate of 1.43% in the feces of humans with enteritis. The isolation rate of *C. jejuni* in humans was lower in our study than in other studies performed in other cities. These differences may be due to variances in consumption rates and cooking procedures for meat, number of examined samples, direct contact with domestic animals, and milk and water hygiene in Kayseri.

Fla-typing methods are applied to understand the epidemiology of campylobacteriosis, and in particular to establish sources of outbreaks and transmission routes. The restriction enzymes used in PCR-RFLP fla-typing methods, such as *Alu*I, *Dde*I, *Hin*fI, *Mbo*I, *Eco*RI, and *Pst*I, are generated from different PCR product fragments and used in various combinations (4,27).

Lindstedt et al. (28) performed *Dde*I digestion on 84 *C. jejuni* strains of different origins and observed 18 different band patterns. In other studies, 19 differently and 19 similarly originated *C. jejuni* isolates were analyzed with RFLP and digested with *Dde*I. The differently originated strains revealed 6 band patterns and similarly originated strains displayed 5 band patterns. The patterns ranged from 3 to 7 bands. However, when digestion was performed with *Hin*fI, fewer bands formed with *Dde*I (29). Ertaş et al. (30) recorded 57 *C. jejuni* strains isolated from broiler chicken carcasses, which formed into 7 different band patterns after fla-typing.

Nielsen et al. (5) typed 80 *C. jejuni* strains (isolated from humans, cattle, and chickens) using 6 different genotyping methods, including PCR-RFLP with *Dde*I and *Alu*I, and detected 40 different band patterns. Harrington et al. (11) reported that *Dde*I appears to provide the best discrimination level, which can be enhanced by combining *Dde*I with *Hin*fI patterns.

*Dde*I and *Hin*fI enzymes were used independently in the present study. After digestion by *Dde*I, strains formed bands ranging from 4 to 7, resulting in 20 different band profiles. No similarities were detected among the band profiles of isolates with different origins. After the completion of RFLP, human, dog, cattle, and chicken isolates were separated into 4, 5, 5, and 6 groups, respectively, according to their banding patterns. However, when isolates were restricted with *Hin*fI, 2 different banding patterns consisting of 2 to 3 bands each were observed. Results of the present study are in agreement with those of previous studies of PCR-RFLP fla-typing (11,31). Harrington et al. (11) also emphasized that *Hin*fI alone was not very discriminatory.

In conclusion, the PCR-RFLP fla-typing method demonstrated particular usefulness for subtype identification of *C. jejuni* isolates. It was observed that the use of enzyme combinations in these techniques provides more information of strain genotyping levels and assists in the understanding of the epidemiological surveillance of strains. It should be noted that the prevalence of *C. jejuni* is high in Kayseri, especially in chicken carcasses, dogs, and cattle.

References

1. Yogasundram, K., Shane S.M., Harrington, K.S.: Prevalence of *Campylobacter jejuni* in selected domestic and wild birds in Louisiana. Avian Dis., 1989; 33: 664-667.
2. Aydın, F., Atabay, H.I., Akan, M.: The isolation and characterization of *Campylobacter jejuni* subsp. *jejuni* from domestic geese (*Anser anser*). J. Appl. Microbiol. 2001; 90: 637-642.
3. Butzler, P.J.: *Campylobacter*, from obscurity to celebrity. Clin. Microbiol. Infect., 2004; 10: 868-876.
4. Wassenaar, T.M., Newell, D.G.: Genotyping of *Campylobacter* spp. Appl. Environ. Microbiol., 2000; 66: 1-9.
5. Nielsen, E.M., Engberg, J., Fussing, V., Petersen, L., Brogren, C.H., On, S.L.W.: Evaluation of phenotypic and genotypic methods for subtyping *Campylobacter jejuni* isolates from humans, poultry and cattle. J. Clin. Microbiol., 2000; 38: 3800-3810.
6. Diker, K.S.: Koyun ve sığırlardan izole edilen *Campylobacter* türlerinin identifikasyonu üzerine çalışmalar. Doğa Bilim Derg. 1985; 9: 232-240.
7. Diker, K.S., Yardımcı, H.: Tavuklardan Campylobacter türlerinin izolasyon ve identifikasyonu üzerinde çalışmalar. TÜBİTAK-VHAG, 671 nolu proje.1987.
8. Gonzalez, I., Grant, K.A., Richardson, P.T., Park, S.F., Collins, M.D.: Specific identification of the enteropathogens *Campylobacter jejuni* and *Campylobacter coli* by using a PCR test based on the *ceuE* gene encoding a putative virulence determinant. J. Clin. Microbiol., 1997; 35: 759-763.
9. CAMPYNET Website. http://www.svs.dk/campynet/
10. Sokal, R.R., Sneath, P.H.A.: Principles of Numerical Taxonomy. W.H. Freeman, San Francisco, 1963; 359 pp.
11. Harrington, C.S., Moran, L., Ridley, A.M., Newell, D.G., Madden, R.H: Inter-laboratory evaluation of three flagellin PCR/RFLP methods for typing *Campylobacter jejuni* and *C. coli*: the CAMPYNET experience. J. Appl. Microbiol., 2003; 95: 1321-1333.
12. Corry, J.E.L., Atabay, H.I.: Poultry as a source of Campylobacter and related organisms. J. Appl. Microbiol., 2001; 90: 96S-114S.
13. Yıldız, A., Diker, K.S.: *Campylobacter* contamination in chicken carcasses. Doğa-Turk J. Vet. Anim. Sci., 1992; 16: 433-439.
14. Kwiatek, K., Wojton, B., Stern, N.J.: Prevalence and distribution of *Campylobacter* spp. on poultry and selected red meat carcasses in Poland. J. Food Prot., 1990; 53: 127-130.
15. Shih, D.Y.: Isolation and identification of enteropathogenic *Campylobacter* spp. from chicken samples in Taipei. J. Food Prot., 2000; 63: 304-308.
16. Quinones-Ramirez, E.I., Vazquez-Salinas, C., Rodas-Suarez, O.R., Ramos-Flores, M.O., Rodriguez-Montano, R.: Frequency of isolation of *Campylobacter* from roasted chicken samples from Mexico city. J. Food. Prot., 2000; 63: 117-119.
17. Özer, D., Ergün, Ö.: İstanbul piyasasında satışa sunulan çeşitli kanatlı eti ve ürünlerinde *Campylobacter jejuni*'nin varlığı üzerine araştırmalar. İstanbul Üniv. Vet. Fak. Derg., 1999; 25: 81-88.
18. Inglis, G.D., Kalischuk, L.D., Busz, H.W.: A survey of *Campylobacter* species shed in faeces of beef cattle using polymerase chain reaction. Can. J. Microbiol., 2003; 49: 655-661.
19. Nielsen, E.M.: Occurrence and strain diversity of thermophilic campylobacters in cattle of different age groups in dairy herds. Lett. Appl. Microbiol., 2002; 35: 85-89.
20. Torre, E., Tello, M.: Factors influencing fecal shedding of *Campylobacter jejuni* in dogs without diarrhea. Am. J. Vet. Res., 1993; 54: 260-262.
21. Sandberg, M., Bergsjo, B., Hofshagen, M., Skjerve, E., Kruse, H.: Risk factors for *Campylobacter* infection in Norwegian cats and dogs. Prev. Vet. Med., 2002; 55: 241-253.
22. Diker, K.S., İstanbulluoğlu, E.: Sağlıklı ve sürgünlü hayvanlarda *Campylobacter fetus* subsp. *jejuni* izolasyonu üzerinde çalışmalar. Ankara Üniv. Vet. Fak. Derg., 1983; 30: 28-34.
23. Cetin, C., Aytuğ, N., Kennerman, E.: İshalli ve sağlıklı buzağılarda *Campylobacter jejuni* ve *Campylobacter coli* prevalansı. Uludağ Üniv. Vet. Fak. Derg., 2000; 19: 9-13.
24. Işık, K., Köse, Ş., Esen, N.: Gastroenteritlerde *Campylobacter jejuni* araştırması. İnfeksiyon Derg., 1996; 10: 337-338.
25. Yıldırım, M.S., Sümerkan, B., Fazlı, Ş.A.: Dışkı kültürlerinden izole edilen *Campylobacter* türlerinin antimikrobiyal ajanlara duyarlılıkları. ANKEM Derg., 1996; 10: 393-398.
26. Aktaş, O., Tuncel, E.: Diyareli hastalarda *Campylobacter jejuni* yönünden bir araştırma. Mikrobiyol. Bült., 1987; 21: 79-85.
27. Kiuchi, A., Hara, M., Pham, H.S., Takikawa, K., Itoh, R., Tabuchi, K.: Detection and investigation of *Campylobacter jejuni* by polymerase chain reaction-restriction fragment length polymorphism analysis. Microbios., 2000; 102: 159-164.
28. Lindstedt, A.B., Heir, E., Vardund, T., Melby, K.K.: Kapperud, G.: Comparative fingerprinting analysis of *Campylobacter jejuni* subsp. *jejuni* strains by amplified-fragment length polymorphism genotyping. J. Clin. Microbiol., 2000; 38: 3379-3387.
29. Petersen, L., Newell, D.G.: The ability of fla-typing schemes to discriminate between strains of *Campylobacter jejuni.* J. Appl. Microbiol., 2001; 91: 217-224.
30. Ertaş, H.B., Çetinkaya, B., Muz, A., Öngör, H.: Genotyping of broiler-originated *Campylobacter jejuni* and *Campylobacter coli* isolates using fla-typing and random amplified polymorphic DNA methods. Int. J. Food Microbiol., 2004; 94: 203-209.
31. Owen, R.J., Leeton, S.: Restriction fragment length polymorphism analysis of the *flaA* gene of *Campylobacter jejuni* for subtyping human, animal and poultry isolates. FEMS Microbiol. Lett., 1999; 176: 345-350.