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RESEARCH ARTICLE

Antibiotic Resistance To *Campylobacter* spp. Isolated from The Livers of Slaughtered Ruminants and Aborted Ovine Fetuses

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ABSTRACT

The presence of *Campylobacter* spp. obtained from clinically healthy 50 sheep, goats and cattle slaughtered in slaughterhouses in Afyonkarahisar and Kutahya provinces and 44 aborted ovine fetuses obtained from the same region was investigated in this study. The subtypes were isolated by culture methods and identified by API Campy (Biomerieux, France) test kits. *Campylobacter* spp. was isolated from 7 samples (15.91%) out of 44 aborted ovine fetuses. After identification of *Campylobacter* spp., it was determined that 5 samples (71.43%) out of 7 were *C. fetus subsp. fetus* (71.43%) and 2 samples were *C. jejuni* (28.57%). Out of the 50 liver samples, *Campylobacter* spp. was isolated from 3 ovine livers (6%) and 1 goat liver (2%). Three of the isolates were identified as *C. jejuni* (75%) and 1 isolate as *C. coli* (25%). *Campylobacter* spp. was not isolated from cattle livers. Resistance rates for ciprofloxacin and tetracycline were 45.5% and 27.3% for erythromycin and 9.1% for ampicillin. No resistance was determined against gentamycin, chloramphenicol and streptomycin. The rate of susceptibility to antibiotics used was 72.7% for ampicillin, 54.5% for erythromycin, 90.9% for gentamycin, 100% for chloramphenicol, 72.7% for streptomycin and 27.3 % for ciprofloxacin and tetracycline.

Keywords: Antibiotic resistance, *Campylobacter* spp., Ovine fetus, Ruminant liver.

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Mezbahalarda Kesilen Ruminant Karaciğerlerinden ve Koyun Abortuslarından İzole Edilen Campylobacter Türlerine Karşı Antibiyotik Dirençliliği

ÖZ

Bu çalışmada, Afyonkarahisar ve Kütahya illerinden mezbahalarda sağlıklı olarak kesilen koyun, keçi ve sığırlardan alınan 50'şer adet karaciğer örneği ile yine aynı bölgeden temin edilen 44 aborte koyun fetüsünde, *Campylobacter* spp. varlığı arandı. Kültür tekniğiyle izole edilen suşlar, API Campy (Biomerieux, France) test kitleriyle identifiye edildi. İncelenen 44 aborte koyun fetüsünden, 7 adet (%15,91) *Campylobacter* spp. izole edildi. İdentifikasyonları yapıldığında, örneklerin 5'inin *C. fetus subsp. fetus* (%71,43), 2'sinin *C. jejuni* (%28,57) olduğu tespit edildi. Ellişer karaciğer örneğinden, 3 koyun karaciğerinde (%6) ve 1 keçi karaciğerinde (%2) *Campylobacter* spp. izole edildi. İzolatların 3'ünün *C. jejuni* (%75), 1'inin *C. coli* (%25) olduğu tespit edildi. Sığır karaciğerlerinden ise *Campylobacter* spp. izole edilemedi. Elde edilen 11 adet *Campylobacter* izolatının analizinde siprofloksasin ve tetrasikline %45,5, eritromisine %27,3, ampisiline %9,1 oranında dirençlilik tespit edildi. Gentamisin, kloramfenikol ve streptomisine karşı ise dirençlilik gözlenmedi. Kullanılan antibiyotikler için tespit edilen duyarlılık oranları, ampisiline %72,7, eritromisine %54,5, gentamisine %90,9, kloramfenikole %100, streptomisine %72.7, siprofloksasin ve tetrasikline %27,3 olarak bulundu.

Anahtar Kelimeler: Antibiyotik direnci, *Campylobacter* spp., Koyun fetüs, Ruminant karaciğer

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INTRODUCTION

Campylobacter species are pathogenic microorganisms for animals and humans which can be commensal in the intestinal flora of various domestic and wild animals and can cause gastrointestinal and genital infections (Songer and Post, 2005).

Generally, the optimal growing temperature is 37°C. However, while thermophilic ones reproduce optimally at 42-43°C, they can also reproduce at 37°C. *C. jejuni, C. coli, C. lari* species are defined as thermophilic species. *Campylobacter* species are microaerophilic. Media containing 5% O_2, 10% CO_2 and 85% N_2 (Moore et al. 2005) is required for optimal reproduction.

Heat-sensitive *Campylobacter* species are inactivated at 60°C in solid and liquid foods in a short time. It has been reported that the *Campylobacter* species is inactivated under standard conditions in chlorinated drinking water and pasteurized milk (Obiri-Danso et al. 2001).

It was observed that *C. jejuni* which has been in the water for a long time passes into the coccoid form and enters into the stagnation phase and that it was not possible to cultivate it by using non-selective media in this phase. It was reported that during this time it is alive but transposed into the coccoid form that cannot be cultured and this form is described as viable but non-culturable (VBNC) (Rolling and Colwell, 1986, Jones et al. 1991, Purdy et al. 1999).
All domestic animals, humans and mammals as well as poultry are hosts. They have no specificity according to animal species. The most common form of a *Campylobacter* infection is enteritis. The course of the disease may range from a mild short course of enteritis to bloody ulcerative colitis. (Aydın et al. 2006).

Campylobacter species are generally susceptible to macrolides and quinolone group antibiotics and have been reported to be resistant to trimethoprim with cephalosporin group antibiotics such as cephalothin, cefoperazone, cefazolin. *C. lari* strains are resistant to nalidixic acid and are used to diagnose and differentiate it from other thermophilic species (Winn et al. 2006).

The antibiotic resistance observed in *Campylobacter* species develops through mutations in chromosomes and from other bacteria through plasmid or transposon transfer (Taylor and Courvalin, 1988).

Campylobacter species is a zoonosis and therefore it is epidemiologically closely related to food and public health and animal health. *Campylobacter fetus subsp. fetus* is epidemic in sheep abortus, causes sporadic abortion in cattle and sporadic infections in other animals. The agent can be active in up to 50% of the intestines and gall bladders of healthy cattle and sheep. During abortion cases, the rate of the agent in stool increases. Feces of infected animals spread into the environment through aborted fetuses and genital discharge (Aydın et al. 2006). Venereal infection is observed in cattle. Following the infection, sheep gain immunity that lasts 2-3 years. Epidemics show a recurrent periodic temporal distribution every 2-3 years (Ülgen 2002).

Campylobacter fetus subsp. venerealis causes abortion and infertility with venereal infection specific to cattle. The agent can be isolated from the distal urethra and glans penis of infected bulls. Accumulation in the female genital tract results in the ascending colonization towards the fallopian tubes. This usually occurs in the form of infertility and abortus occurs in less than 10% of infected cows (Songer and Post, 2005).

Infectious diseases causing abortion in sheep; Brucellosis, Campylobacteriosis, Salmonellosis, Chlamydiosis, Listeriosis, Leptospirosis, Toxoplasmosis and Aspergillosis (Kenar et al. 1990).

Ovine campylobacteriosis is a disease, which can cause severe economic losses due to abortions (Diker and Istanbulluoglu, 1986). The Epidemiology of sheep abortion due to Campylobacter species is similar to other sheep abortion infections (Ülgen 2002, Aydın et al. 2006, Batmaz 2013).

More precise and faster techniques have been developed in order to identify and determine the genetic characteristics of the *Campylobacter* species. In addition to biochemical tests, techniques such as Polymerase Chain Reaction (PCR), Immunomagnetic Separation (IMS), API Campy Test Kit, Polymorphic DNA (RAPD), Pulsed-Field Gel Electrophoresis (PFGE) are being used. Thus, in addition to time savings, identifications can be made more precisely (Lamoureux et al. 1997, Shih 2000, Madigan et al. 2003).

The objective of this study was to investigate the presence of *Campylobacter* species which is an important pathogen in terms of animal and public health, in aborted fetuses and sheep, goat and bovine livers and determine the antibiotic resistance of the obtained strains.

MATERIAL and METHOD

The study was carried out on the livers of sheep, goats, cattle slaughtered in slaughterhouses in Afyonkarahisar and Kütahya provinces in Midwest Anatolia and the fetuses from aborted sheep. 10 ml of the abomasum content of 44 sheep fetuses freshly supplied from abortus cases incurring in April 2015 -

May 2016 in the said environment was obtained by means of a sterile injector from each fetus. In the period of January 2016 - May 2016, 50 sheep, goat and bovine livers from healthy animals slaughtered in slaughterhouses were collected by transferring them to sterile specimen containers with the help of a sterile scalpel and scissors. The samples were transferred to the laboratory under a cold chain. Forty-four fetuses were collected from 38 sheep farms and a total of 194 samples including 50 liver samples from sheep, goats and cattle were used.

The isolation of *Campylobacter* species was carried out by using the following media and supplements: Nutrient Broth No. 2 (Oxoid CM 0067), Selective Supplement (Oxoid SR 048). Charcoal Cefaperazone Deoxycholate Agar (CCDA), (Oxoid CM 0739), CCDA Selective Supplement (Oxoid SR0155E).

The identification of *Campylobacter* species was carried out by using the following tests: Oxidase Test, Oxidase Identification Sticks (Oxoid BR 064), Catalase Test, 3% hydrogen peroxide (Merck 8597) and hippurate hydrolysis test (Merck-8.20648.0025), API Campy Test.

The tests were carried out according to the manufacturer's instructions. Isolated *Campylobacter* spp were cultivated on 5-7% sheep blood Mueller – Hinton Agar (Oxoid CM0337) media and disks with Ampicillin (Oxoid CT003B) 10 μg, Erythromycin (Oxoid CT0020B) 15 μg, Gentamicin (Oxoid CT0024B) 10 μg, Chloramphenicol (Oxoid CT0013B) 30 μg, Streptomycin (Oxoid CT0047B) 10 μg, Ciprofloxacin (Oxoid CT0425B) 5 μg, Tetracycline (Oxoid CT0054B) 30 μg were placed on the agar (Arda et al. 1987).

The samples were taken in sterile containers to the laboratory under a cold chain and isolation and identification procedures were started on their delivery date. The medium containing 5% O2, 10% CO2 and 85% N2 was kept at 37°C for 3-7 days. Their proliferation was checked. The *Campylobacter* species were left to incubate at 37°C for 48-72 hours in a microaerophilic medium.

Antibiotic susceptibility was assessed according to Clinical and Laboratory Standards Institute (CLSI) Performance standards for antimicrobial susceptibility testing (2012).

RESULTS

Campylobacter spp. was determined in 11 (5.67%) out of the total 194 samples. *Campylobacter* spp. was determined in 7 of the examined 44 lamb abomasum (15.91%), in 3 of the examined 50 sheep livers (6%) and 1 (2%) of the 50 goat livers. No isolation was made from bovine livers.

63.64% (7) of the samples which revealed the presence of *Campylobacter* spp. were isolated from sheep fetuses, 27.27% (3) from sheep livers and 9.09% (1) from goat liver samples.

5 out of the 11 isolates were identified as *Campylobacter fetus ssp. fetus* (45.45%), 5 were identified as *Campylobacter jejuni* (45.45%) and 1 was identified as *Campylobacter coli* (9.09%).

5 out of the 7 fetus samples found positive were identified with *Campylobacter fetus ssp. fetus* (71.43%), 2 samples with (28.57%) *Campylobacter jejuni,* 2 out of 3 sheep liver samples revealed (66.67%) *Campylobacter jejuni,* 1 had *Campylobacter coli* while 1 goat liver sample was identified with *Campylobacter jejuni.* The distribution of the identified *Campylobacter* species according to the samples is shown in Table 1.

Resistance to 7 different antibiotics for the identified 11 isolates was determined by the disc diffusion method. According to the test results the highest resistance was manifested against ciprofloxacin and tetracycline (45.45%) while the highest sensitivity was against gentamicin (90.91%) and chloramphenicol (100%). Resistance and sensitivity data against determined antibiotics and strains is shown in Table 2.

Table 1. Distribution of the identified *Campylobacter* species according to the samples

Agent	Sheep				Goat liver		Bovine liver		Total samples	
	Fetus n=44		Liver n=50		n=50		n=50			
	x	%	x	%	x	%	x	%	194	%
C. fetus subsp. fetus	5	11.36	-	0.00	-	-	-	-	5	2.58
C. jejuni	2	4.55	2	4.00	1	2.00	-	-	5	2.58
C. coli	-	-	1	2.00	-	-	-	-	1	0.52
Total	7	15.91	3	6.00	1	2.00	0	0	11	5.67

(x: number of positive samples, n: number of examined samples)

Table 2. *Campylobacter* spp. Antibiogram Results

Antibiotic	S		I		R	
	n	%	n	%	n	%
Ampicillin	8	72.73	2	18.18	1	9.09
Erythromycin	6	54.55	2	18.18	3	27.27
Gentamicin	10	90.91	1	9.09	-	-
Chloramphenicol	11	100.00	-	-	-	-
Streptomycin	8	72.73	3	27.27	-	-
Ciprofloxacin	3	27.27	3	27.27	5	45.45
Tetracycline	3	27.27	3	27.27	5	45.45

(n: Number of strains, S: sensitive, I: medium sensitive, R: resistant)

DISCUSSION

The study revealed that 11 (5.67%) samples were positive for Campylobacter spp. Resistance was manifested against ciprofloxacin and tetracycline in the identified isolates while sensitivity to gentamicin and chloramphenicol was determined. Campylobacteriosis is a common zoonotic disease throughout the world and observed in Latin America, Asia, Africa, North America, Europe and New Zealand (Gard 2016). The incidence of infections caused by *Campylobacter* spp. is constantly increasing. Campylobacteriosis is a zoonosis infection. *Campylobacter* spp. causes hundreds of millions of infections around the world every year (Kashoma et al 2015). The disease is also common in our country. Kenar and Erganiş (1994) carried out a study in Samsun and its periphery regarding *Campylobacter* induced abortions and reported that they had isolated *Campylobacter* subsp in 8 out of 35 aborted lamb fetuses (22.9%) and 5 of them were identified as *C. fetus subsp. fetus* (62.5%), 2 as (25.0%) *C. jejuni* and 1 as Aerotolerant campylobacter. Similarly, in a study carried out by Kenar et al. (1990) in the region of Konya they reported that they had isolated *Campylobacter* spp. at a rate of 7.5% in aborted fetuses. Küçükayan et al. (2003-2007) carried out a study and reported that they had isolated 6 of the examined 463 fetuses (1.29%) with *Campylobacter* spp. and all of them were diagnosed with *C. fetus subsp. fetus*. In a study carried out by Sağlam et al. (1998) in Northeast Anatolia they reported that 5 out of 119 (4.2%) aborted sheep fetuses were identified with *C. fetus subsp. fetus* ssp. In a previous study in the same region (Aydın et al. 1994), the isolation rate was reported as 31.25%. Likewise, in a study carried out by Arda et al. (1987) in the Central Anatolian Region, they reported that they had isolated and identified *C. fetus subsp. fetus* in 13 out of 173 aborted fetuses (7.5%). In this study, *Campylobacter* spp. was isolated in 7 (15.91%) out of 44 aborted sheep fetuses. The values were evaluated as compatible with other studies.

In a study carried out with sheep and goat liver surface swabs (Lazou et al. 2014a) it was reported that 44 (44%) of 100 samples were isolated with *Campylobacter* spp. In another study (Lazou et al. 2014b) by the same researchers they reported that 78,2% of the liver surfaces collected from lambs, sheep, goats and kids slaughtered in slaughterhouses had been determined as contaminated with *Campylobacter* agents. *Campylobacter* spp. was isolated in 3 (6%) sheep livers and 1 (2%) goat liver in the current study. The researchers think that the difference between the results of this study and the results of the others can be attributed to the difference in the course of the disease in the regions where the studies were carried out, the fact that the samples were collected when the infection was peaking or the difference in diagnostic techniques. Furthermore, the difference in the results could be attributed to the fact that the reported studies had used lambs and kids while we used sheep and goats in our study. *Campylobacter* spp. agents are more common in young animals. *Campylobacter* spp. could not be isolated from the bovine liver samples in the present study. In our country, Elmalı (2004) has reported isolation of 8% in liver samples taken from cattle and Açık (2006) has reported isolation of 6.5%. Enokimoto et al. (2007) reported that 5% of 108 cattle liver samples revealed *Campylobacter* spp., yet the isolation was 45% from bile samples taken from the same cattle. Matsumoto et al. (2008) reported that they had isolated *C. jejuni* at a rate of 1.4% in their study. Unlike the reported studies, the fact that *Campylobacter* could not be isolated in the current study can be attributed to the good hygienic conditions of the slaughterhouses where the samples were taken. This can be attributed to the influence of the more effective inspection of the production of food of animal origin initiated in our country by the Ministry of Food, Agriculture and Livestock in recent years, the noticeable increase of technical and hygienic standards in the abattoirs during the study as well as the reduction of cross contamination of the

carcass and internal organs caused by bile and stool contamination.

It was observed that the *Campylobacter* species isolated in our study were susceptible to chloramphenicol, gentamicin, ampicillin and streptomycin and less sensitive to erythromycin. The isolated *Campylobacter* species were resistant to ciprofloxacin and tetracycline. Hakkinen et al. (2007) reported resistance to ampicillin and tetracycline, but no resistance to erythromycin in their study. In their study on *Campylobacter* species isolated from cattle livers, Aneesa and Fakr (2013) reported sensitivity against erythromycin, gentamicin, chloramphenicol, streptomycin and medium level sensitivity against ampicillin, ciprofloxacin and tetracycline. Karikari et al. (2017) reported that the *Campylobacter* species they had isolated from cattle carcasses were resistant to erythromycin, ampicillin, chloramphenicol, sensitive to gentamicin and moderately sensitive to ciprofloxacin and tetracycline. The reason for the differences between the results of the studies mentioned above and the results presented in this study can be attributed to the differences in the usage rate and duration of the antibiotics in the investigated countries.

It has been concluded that the isolation rates of *Campylobacter* species may vary according to the regions where the study is conducted and that the antibiotic sensitivities may be different according to the regions and furthermore, that the improvement in the technical and hygienic conditions in slaughterhouses in our country in recent years may be effective in the isolation of *Campylobacter*.

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