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Isolation, identification and antibiotic susceptibility of Campylobacter spp. in aborted sheep fetuses*)

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Summary

The aim of this study was to isolate, identify and determine the antibiotic susceptibility of Campylobacter spp in aborted sheep fetuses in Diyarbakır and its vicinity. The bacteria were isolated by conventional methods. Biochemical, growth and tolerance characteristics of the organism were used to identify the isolates. A total of 100 aborted fetuses from 25 different locations were investigated and 10 (10%) yielded Campylobacter spp. Of the isolated bacteria, 7 (7%) were C. fetus subsp. fetus and 3 (3%) were C. jejuni. All isolates were resistant to penicillin and susceptible to enrofloxacin; 9 isolates were intermediately susceptible to streptomycin, 8 isolates were intermediately susceptible to tetracycline and susceptible to gentamycin and neomycin and 7 isolates were susceptible to erythromycin.

Keywords: sheep, abortion, Campylobacter spp., antibiotic susceptibility

Complications due to infection with *Campylobacter* spp. in sheep is a major problem in sheep breeding in Turkey as well as throughout the world. Particularly in developed countries where brucellosis is no longer a problem, campylobacteriosis has become the major cause of abortion among sheep (18, 21).

Campylobacter spp., is a zoonotic microorganism that resides in the gastrointestinal system of animals as a commensal organism. In addition to abortive infections among pregnant sheep, it causes gastrointestinal system infections accompanied by diarrhea. Campylobacter infections among sheep are highly contagious and are characterized by abortion in the last trimester of pregnancy, stillbirth, premature birth and lambs with low birth weight (3, 4). Campylobacter infections in sheep have specific epidemiological and pathogenetic characteristics. Presence of *Campylobacter* spp. in the gastrointestinal tract of healthy animals is an uncommon characteristic for other agents causing abortion. *Campylobacter* spp. are transmitted to humans by food such as meat and milk contaminated with animal feces, and causes infections with high fever of unknown origin, gastrointestinal and genital system infections, meningitis, arthritis and endocarditis (23).

The infection is transmitted to susceptible sheep by the feces of infected animals, wild birds, cattle, unprocessed water, and from feeding in pastures contaminated with aborted fetuses and genital discharges. Studies suggest that the major route of transmission among sheep is the oral route. There is no venereal transmission among sheep (3, 4, 23). *C. fetus* subsp. *fetus*, *C. jejuni* and *C. coli* have been isolated from aborted fetuses in sheep (6, 8, 21).

Various techniques such as serology, polymerase chain reaction (PCR), immunofluorescence and immunoperoxidase staining may also be used to detect *Campylobacter* spp. (3, 4, 7). We isolated and identified *Campylobacter* spp. by a conventional method preferred in many laboratories (25).

The aim of this study was to isolate *Campylobacter* spp. from aborted sheep fetuses, determine the epidemiological significance of this infection, identify isolates by morphologic, biochemical, growth and tolerance characteristics, and to determine their antibiotic susceptibilities.

Material and methods

Material. A total of 100 aborted fetuses collected from Diyarbakır and its vicinity and delivered to veterinary clinics during the lambing season of 2001-2003, were used as samples.

Collection of samples and Campylobacter isolation. Visceral organs (lungs, liver, kidney, heart, spleen) and stomach contents of aborted fetuses were aseptically extracted and used for bacteriological examinations. Samples were kept in sterile jars containing saline with glycerin and were

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Tab. 1. The origin of *Campylobacter* spp. number isolated and identified species

Location	No. of samples	No. of isolation	Species of identificated
Karaçimen	6	2	*C. fetus* subsp. *fetus*
Mermer	3	1	*C. fetus* subsp. *fetus*
Eryolu	2	1	*C. fetus* subsp. *fetus*
Bati Karakoç	4	1	*C. fetus* subsp. *fetus*
Şilbe	2	2	*C. jejuni*
Fabrika	3	1	*C. fetus* subsp. *fetus*
Çınar merkez	5	1	*C. fetus* subsp. *fetus*
Bağivar	3	1	*C. jejuni*

transported to the Microbiology Laboratory of the Medical Faculty, Dicle University, within 1-2 hours. Table 1 shows the origin and numbers of samples examined.

Each visceral organ was divided into small pieces with sterile forceps and scissors. Tissue pieces were inoculated in 2 ml of Preston broth enrichment medium. Inoculated specimens were incubated at 37°C under microaerophilic conditions for 24 hours. At the end of the incubation period, 0.5 ml was drawn from each sample and inoculated on Campylobacter Agar Base (Oxoid CM689) enriched with 7% horse serum and supplemented with Skirrow supplement (Oxoid SR69) (3, 4).

Inoculated plates were incubated at 37°C under microaerophilic conditions for 3-5 days. To create a microaerophilic environment, Campy-Gen kits (Oxoid CN25) were placed in anaerobic jars (3, 4).

Microscopic features. Jars were opened at the end of the incubation period and the colonies on the plate were examined to determine *Campylobacter* spp. Preparations were made from colonies suspected to be *Campylobacter* spp and they were Gram stained. Motile microorganisms with a characteristic morphology such as curved, gull-winged, 'S'-shaped or spiral forms, were considered to be *Campylobacter* spp. (3, 4).

Identification procedure. Hippurate hydrolysis test, catalase and oxidase tests, growth under various temperatures, salt tolerance test, nalidixic acid (NA), cephalotin (CN) susceptibility test, and glycine tolerance test were performed to identify isolates suspected to be *Campylobacter* spp. (20, 25).

Antibiotic susceptibility testing. Susceptibility testing was performed on Mueller-Hinton agar enriched with 7% defibrinated sheep blood. Isolates to be tested were grown in brucella broth for 18-24 hours to yield a Mac Farland value of 0.5; 0.2 ml of specimen for each isolate was inoculated on agar with a glass rod. Afterwards antibiotic disks (enrofloxacin, streptomycin, neomycin, tetracycline, penicillin, gentamycin, and erythromycin) were placed on the surface of the agar. The plate was kept in the refrigerator for 30 minutes with the lid up. This was then incubated at 37°C under microaerophilic conditions for 24 hours. Upon completion of the incubation period, the surface of the agar was checked routinely until a visible growth appeared. The zone diameters were measured. Results obtained were assessed as resistant, intermediately susceptible, and susceptible according to standards (2).

Results and discussion

Table 1 shows the origin of isolated *Campylobacter* spp., number of isolates and the identified *Campylobacter* spp. Of the 100 aborted fetuses, 10 (10%) yielded *Campylobacter* spp. with 7 (70%) identified as *C. fetus* subsp. *fetus* and 3 (30%) as *C. jejuni*.

The results of the antibiotic susceptibility tests of *Campylobacter* spp. isolated from aborted fetuses are shown in tab. 2.

While all isolates were resistant to penicillin, 9 were intermediately susceptible to streptomycin, 8 were intermediately susceptible to tetracycline as well as to gentamycin and neomycin, and 7 were susceptible to erythromycin. All isolates were susceptible to enrofloxacin.

Campylobacter spp. have recently become the center of interest and research.

Diker (9) isolated 15 *C. fetus* subsp. *fetus* out of 124 (12%) aborted sheep fetuses. Kenar et al. (14) reported 20 *Campylobacter* spp. isolates out of 303 (7.5%) aborted sheep fetuses. Muz et al. (19), identified 5 *C. fetus* subsp. *fetus* isolates out of 110 (4.5%) aborted fetuses in their study in Elazığ and its vicinity during 1995-1996. Kenar and Erganis (15) investigated 35 aborted sheep fetuses in Samsun and neighboring provinces during the 1991-1992 lambing season; 8 (22.9%) yielded *Campylobacter* spp. Of these, 5 (62.5%) were *C. fetus* subsp. *fetus*, 2 (25%) were *C. jejuni* and 1 (12.5%) was aerotolerant *Campylobacter*. Erdogan et al. (10) reported the isolation of 4 *C. fetus* species out of 145 aborted sheep fetuses during 1989-1992.

The isolation rate of *Campylobacter* spp. (*C. fetus* subsp. *fetus*, *C. jejuni* and *C. coli*) in bacteriological examinations on sheep fetuses in Turkey is 1.2-12% (9, 5).

Tab. 2. Antibiotic susceptibility testing results of isolates

Antimicrobial agent	C.f.1	C.f.2	C.f.3	C.f.4	C.f.5	C.f.6	C.f.7	C.j.1	C.j.2	C.j.3
Penicillin	R	R	R	R	R	R	R	R	R	R
Streptomycin	I	I	R	I	I	I	I	I	I	I
Tetracycline	I	I	I	R	I	I	I	S	I	I
Gentamycin	S	I	S	S	S	R	S	S	S	S
Neomycin	S	S	I	S	I	S	S	S	S	S
Erythromycin	S	S	S	S	I	I	S	S	S	I
Enrofloxacin	S	S	S	S	S	S	S	S	S	S

Explanation: C.f. – *Campylobacter fetus*; C.j. – *Campylobacter jejuni*; R – rezistant; I – intermediate; S – sensitiv

In a study by Hansen et al., (13) in the United States, the risk of abortion due to *Campylobacter* species was reported to be 5-17%. Latinoviç et al. (17) reported an abortion rate of 15.2% among sheep due to *Campylobacter* spp. in Yugoslavia, mostly attributed to *C. fetus* and *C. jejuni*, respectively. A study by Allsup (1) in the United Kingdom suggested that campylobacteriosis was the third most common entity responsible for abortion in sheep. In the same study, Allsup indicated that the abortion rate due to campylobacteriosis had changed from 6.8% in 1982 to 13.1% in 1984. The most common species were *C. jejuni* biotype 1-2, *C. fetus* subsp. *fetus* and *C. coli*.

Diker (9) tested the antibiotic susceptibility of 100 *C. jejuni* isolates and found that the highest rate of resistance (81%) was to penicillin and the highest rates of sensitivity in decreasing order were to gentamycin (93%), chloramphenicol (91%) and erythromycin (90%); sensitivity to kanamycin and tetracycline was 70%. Kenar and Erganis (15) reported that their *C. jejuni*, *C. fetus* and aerotolerant *Campylobacter* isolates were 90% resistant to penicillin, erythromycin, gentamycin, chloramphenicol, trimethoprim-sulphametoxazole and 70% to streptomycin, tetracycline and neomycin. Of the 10 *Campylobacter* spp. isolated in the study, 100% were resistant to penicillin, 9 (90%) were intermediately sensitive to streptomycin, 8 (80%) were intermediately sensitive to tetracycline, 8 (80%) were sensitive to neomycin and gentamycin, and all (100%) were sensitive to enrofloxacine. The results of the presented study are compatible with the data of other studies carried out in Turkey and other countries. Delong et al. (6) isolated 15 *Campylobacter* spp. out of 27 aborted fetuses in the US during the 1992-1993 lambing season. Of these, 14 were identified as *C. jejuni* and 1 as *C. fetus* supsp. *fetus*. Lander (16) stated that although the abortion rate due to campylobacteriosis was usually 15-20%, it sometimes increased to 70%. Erganis et al. (11) isolated 13 *C. fetus* species out of 58 aborted fetuses during 1993-1994. Differences between studies may be attributed to geographical region, diagnostic techniques, collection and timing of materials.

According to antibiotic susceptibility test results, the most effective antibiotics against *Campylobacter* spp. in Turkey were enrofloxacine, gentamycin, neomycin and erythromycin, respectively. Data from national (9, 14) and international (12, 22) studies are compatible.

Conclusion

Abortion due to *Campylobacter* spp. in sheep in the region is a major problem and causes abortion in 10% of animals. Since *Camplyobacter* spp. are zoonotic agents, consumption of sheep meat and milk may lead to the transmission of infection to humans and thus should be seriously considered.

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