Kafkas Univ Vet Fak Derg
25 (5): 697-701, 2019
DOI: 10.9775/kvfd.2018.21654

Kafkas Universitesi Veteriner Fakultesi Dergisi
ISSN: 1300-6045 e-ISSN: 1309-2251
Journal Home-Page: http://vetdergikafkas.org
Online Submission: http://submit.vetdergikafkas.org

Research Article

Isolation and Molecular Identification of *Campylobacter* spp. from Vaginal Swab Sample Obtained from Sheep Herds with Abort History

Aliye GÜLMEZ SAĞLAM [1,a] Doğan AKÇA [2,b] Özgür ÇELEBİ [1,c] Fatih BÜYÜK [1,d]
Elif ÇELİK [1,e] Mustafa Reha COŞKUN [1,f] Mitat ŞAHİN [1] Salih OTLU [1,g]

[1] Department of Microbiology, Faculty of Veterinary Medicine, Kafkas University, TR-36100 Kars - TURKEY
[2] Faculty of Health Sciences, Kafkas University, TR-36100 Kars - TURKEY
[a] ORCID:0000-0002-7639-5075; [b] ORCID:0000-0002-3986-8769; [c] ORCID:0000-0002-3478-008X; [d] ORCID:0000-0003-3278-4834
[e] ORCID:0000-0003-4531-3863; [f] ORCID: 0000-0002-1441-3995; [g] ORCID: 0000-0001-8490-2279

Article Code: KVFD-2018-21654 Received: 27.12.2018 Accepted: 10.04.2019 Published Online: 17.04.2019

How to Cite This Article

Gülmez Sağlam A, Akça D, Çelebi Ö, Büyük F, Çelik E, Coşkun MR, Şahin M, Otlu S: Isolation and molecular identification of *Campylobacter* spp. from vaginal swab sample obtained from sheep herds with abort history. *Kafkas Univ Vet Fak Derg,* 25 (5): 697-701, 2019. DOI: 10.9775/kvfd.2018.21654

Abstract

Campylobacteriosis is a contagious and zoonotic infection characterized by abortion and infertility in cattle and sheep. The objective of this study was to investigate *Campylobacter* spp. cause to abortion in sheep herds in Kars province. For this purpose, a total of 350 vaginal swab samples obtained from sheep with abortion were examined by cultural and molecular methods. Swab samples were inoculated on Preston Campylobacter Selective Agar for isolation of *Campylobacter* species. Following the incubation, suspected colonies were subjected to Gram staining, mobility, oxidase and catalase tests for identification. Multiplex PCR (m-PCR) was used for the identification of *Campylobacter* isolates at species level. *Campylobacter* spp. was isolated in 8 (2.28%) of the 350 vaginal swab samples examined. Of 3 isolates were identified as *Campylobacter jejuni* and 5 were *C. coli* by m-PCR. According to the data obtained from this study, it was revealed that *Campylobacter* species should be taken into consideration in the abortion cases of sheep in this region. Considering the risk of this infection both in terms of animal health and human health, it is thought that more attention should be given to protection and control measures.

Keywords: *Thermophilic Campylobacter spp., Sheep, Vaginal swab, m-PCR*

Abort Öykülü Koyun Sürülerinden Alınan Vaginal Sıvap Örneklerinden *Campylobacter* spp. İzolasyonu ve Moleküler İdentifikasyonu

Öz

Campylobacteriosis, sığır ve koyunlarda yavru atımı ve infertilite ile karakterize, bulaşıcı ve zoonotik bir infeksiyondur. Bu çalışmada, Kars ilindeki koyun sürülerinde gözlenen abort olaylarının *Campylobacter* spp. yönünden araştırılması amaçlandı. Bu amaçla, abort olgularının görüldüğü koyun sürülerinden alınan toplam 350 adet vaginal sıvap örneği kültürel ve moleküler yöntemlerle incelendi. *Campylobacter* türlerinin izolasyonu amacıyla alınan örneklerin Preston Campylobacter Selektif Agara ekimleri yapıldı. Üreme sonucu şüpheli kolonilere identifikasyon amacıyla, Gram boyama ve hareketlilik muayeneleri ile oksidaz ve katalaz testleri uygulandı. *Campylobacter* spp. olarak belirlenen şüpheli kolonilerin tür düzeyinde identifikasyonu için Multiplex PCR (m-PCR) kullanıldı. Toplamda incelenen 350 vaginal sıvap örneğinin 8 (%2.28)'inde *Campylobacter* spp. izolasyonu gerçekleştirildi. Multiplex PCR sonucunda 3'ü *Campylobacter jejuni* ve 5'i *C. coli* olarak tespit edildi. Bu çalışma sonucunda elde edilen verilere bakılarak yöremizdeki koyunlarda meydana gelen atık olgularında *Campylobacter* türlerinin de göz önüne alınması gerektiği ortaya konulmuştur. Bu infeksiyonun hem hayvan sağlığı hem de insan sağlığı açısından oluşturduğu risk göz önüne alınırsa koruma ve kontrol tedbirleri açısından daha fazla önem verilmesi gerektiği düşünülmektedir.

Anahtar sözcükler: *Termofilik Campylobacter spp., Koyun, Vaginal sıvap, m-PCR*

INTRODUCTION

Sheep breeding constitutes a significant part of the animal husbandry of Turkey. According to the data of 2017, the sheep population of Turkey is about 33 million and 450 thousand of which are farming in the Kars region [1]. One of the most important problems encountered in sheep breeding and economically damaging to the breeder is the abortion case. Bacterial, viral and protozoal infections are among the causes of abortion in animals. These

İletişim (Correspondence)
+90 474 2426836
alis_6223@hotmail.com

infections are important in terms of public health as well as economically. Among the bacterial infections, brucellosis, campylobacteriosis, chlamydiosis and salmonellosis are responsible for most cases of abortion [2,3].

Campylobacteria which are pathogenic microorganisms for other animals and humans, can be found commensively in the intestinal flora of various domestic and wild animals and can cause gastrointestinal and genital infections in some cases. *Campylobacter* species cause epidemics in sheep and sporadic infections in other animals. Although healthy sheep can carry the bacteria in the intestine and gallbladder without clinical infection, some *Campylobacter* species can cause systemic infections. *Campylobacter* was first isolated from the aborted sheep fetus. Agent spreads to the environment through feces and genital secretions of infected animal and aborted fetus. When the disease first comes out, abort cases in the herd is seen 60-70%. *Campylobacter* infections are characterized by abortion, stillbirth, birth of premature and poor lambs in the 4-5th month of pregnancy and death of sheep due to metritis [4-8].

Campylobacter jejuni, *C. coli* and *C. fetus* subsp. *fetus* are species that are common in the world and cause reproductive diseases in sheep. The agent is Gram negative, motile and microaerophilic. Environmental samples such as soil and water and food can be contaminated with *Campylobacter* spp. as the result of contact with contaminants such as feces and aborted fetus. It is known that *Campylobacter* species cause cross-infection among some animal species [9]. Roug et al.[10], isolated *C. jejuni* and *C. coli* from sheep, goat, cattle and pigs in agricultural fairs in California. Results of this study are thought to show transmission *Campylobacter* species among animal species. Pao et al.[11], showed that sheep in small ruminant farms were exposed to *C. jejuni* infections at a greater risk than goats. Healthy sheep serve as reservoirs for *Campylobacter* species, especially in stressful conditions such as birth, weaning, and nutritional changes [12,13].

In this study, it was aimed to investigate the *Campylobacter* spp., which is one of the important abortion agents, from vaginal swab samples collected from sheep herds in the Kars region.

MATERIAL and METHODS

Ethical Approval

The experiment was carried out with the approval of Kafkas University Local Ethical Committee for Animal Experiments (KAÜ-HADYEK/2018-114).

Samples

Totally 350 vaginal swab samples obtained from 7 sheep herds in the Kars region were investigated for *Campylobacter* species.

Bacterial Isolation and Identification

In this study, vaginal swab samples were examined by the culture method. For pre-enrichment step, samples were inoculated in Preston Campylobacter Enrichment Broth containing 7% defibrinated horse blood and Preston Campylobacter selective supplement (SR117, OXOID) and were incubated in microaerobic conditions at 37°C and 42°C for 48 h. After incubation, 100 µL of the pre-enriched culture was plated on Preston Campylobacter Selective Agar plates and the plates were incubated at 37°C and 42°C for 48-72 h. The growth cultures were evaluated for the colony morphology, microscopic appearance, catalase and oxidase properties [14,15].

DNA Extraction and Multiplex PCR

The classical phenol-chloroform extraction method [16] was used for DNA extraction from the Campylobacter isolates. Then, the multiplex PCR (m-PCR) technique was carried out for thermophilic *Campylobacter* and the m-PCR was for *C. fetus* and *C. venerealis* [17,18]. The primer sets targeting the 23S rRNA gene of *Campylobacter* spp., the *hipO* gene of *C. jejuni*, the *glyA* gene of *C. coli*, *C. lari*, the *cstA* gene of *C. fetus*, the *virB11* gene of *C. venerealis* were used with the exception of the specific amplified products as 650, 323, 126, 251, 764 and 233 bp respectively *(Table 1)* [17,18]. Both genus and species-specific PCR was conducted in a single reaction.

Each m-PCR tube for thermophilic *Campylobacter* spp. contained 200 µM dNTP (Thermo Scientific, Lithuania); 2.5 µL of 10x reaction buffer (Thermo Scientific, Lithuania), 20 mM $MgCl_2$ (Thermo Scientific, Lithuania); 0.5 µM *C. jejuni* and *C. lari* primers; 1 µM *C. coli* and *C. fetus* primers, 2 µM *C. upsaliensis* primers; 0.2 µM 23S rRNA primer *(Table 1)*; 1.25 U of *Taq* DNA polymerase (Thermo Scientific, Lithuania), and 2.5 µL of whole-cell template DNA. The volume was adjusted with sterile distilled water to give 25 µL. DNA amplification was carried out in a thermocycler (Bio-rad, U.S.A) using an initial denaturation step at 95°C for 6 min followed by 30 cycles of amplification (denaturation at 95°C for 0.5 min, annealing at 59°C for 0.5 min, and extension at 72°C for 0.5 min), and was finalized with an extension at 72°C for 7 min.

Each m-PCR tube for *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis* contained 0.5 mM of each dNTP (Thermo Scientific, Lithuania), 2 µL of 1x reaction buffer (Thermo Scientific, Lithuania), 2.5 mM $MgCl_2$ (Thermo Scientific, Lithuania), 0.625 µM MG3F/MG4R primer set, 0.375 µM nC1165g4F/nC1165g4R primer set, and 1.5 U Taq DNA polymerase (Thermo Scientific, Lithuania), 1 µL of whole-cell template DNA. The volume was adjusted with sterile distilled water to give 20 µL. For amplification, the following cycling conditions were used: initial denaturation for 3 min at 95°C followed by 35 cycles of denaturation for 30 sec at 94°C, annealing for 30 sec at 53°C, and extension for 1 min at 72°C.

Table 1. *Primer sequences used in the multiplex PCR assay and the expected sizes of the amplified products*

Primer	Sequence (5'–3')	Size	Target Gene
23SF 23SR	TATACCGGTAAGGAGTGCTGGAG ATCAATTAACCTTCGAGCACCG	650	23S rRNA
CJF CJR	ACTTCTTTATTGCTTGCTGC GCCACAACAAGTAAAGAAGC	323	*C. jejuni hipO*
CCF CCR	GTAAAACCAAAGCTTATCGTG TCCAGCAATGTGTGCAATG	126	C. coli glyA
CLF CLR	TAGAGAGATAGCAAAAGAGA TACACATAATAATCCCACCC	251	*C. lari glyA*
MG3F MG4R	GGTAGCCGCAGCTGCTAAGAT TAGCTACAATAACGACAACT	764	*C. fetus cstA*
nC1165g4F nC1165g4R	AGGACACAAATGGTAACTGG GATTGTATAGCGGACTTTGC	233	*C. fetus* subsp. *venerealis* virB11

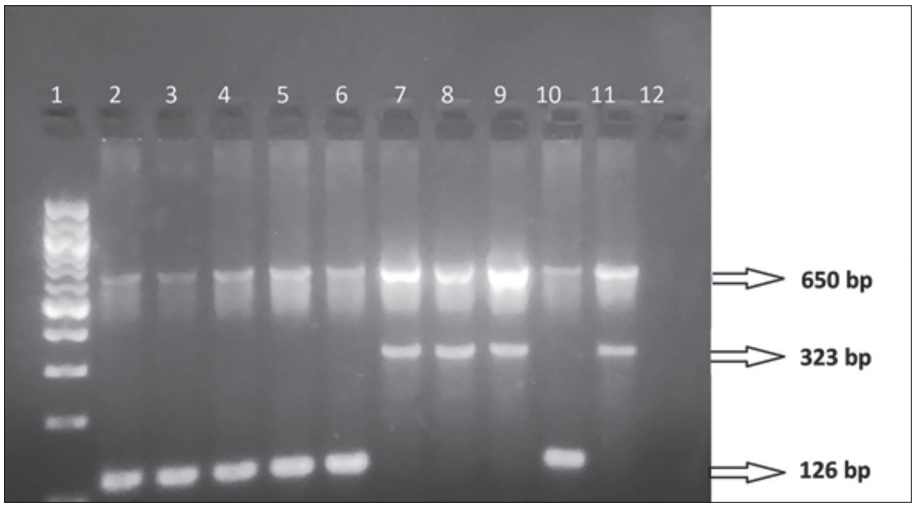

Fig 1. Gel electrophoresis image of m-PCR for thermophilic *Campylobacter* species. 1: DNA marker (Gene ruler 100 bp DNA Ladder, Fermentas); 2-9: Positive samples; 10: Positive control for *C. coli;* 11: Positive control for *C. jejuni;* 12: Negative control

The PCR reaction is accompanied by the *Campylobacter* reference strains and the amplified products were visualized by 1.5% agarose gel electrophoresis and the images were photographed under UV transilluminator (UVP, CA 91786, U.S.A.).

RESULTS

As the result of cultural examination colonies of the *Campylobacter* spp. were isolated showing microscopic characteristics such as small size, pinpoint morphology, non-hemolytic, and Gram-negative "gull-wing" shaped bacilli. Suspected isolates were subjected to biochemical tests. Thus, *Campylobacter* spp. was isolated in 8 (2.28%) of the 350 vaginal swab samples. Eight isolates, which were characterized as *Campylobacter* spp., were identified as *C. coli* (in 5 isolates) and *C. jejuni* (in 3 isolates) by using species-specific m-PCR *(Fig. 1)*.

DISCUSSION

Sheep farming has great importance for husbandry in Turkey. Abortions caused by infectious agents in sheep breeding are an important problem. These agents lead to significant economic losses, not only to a loss of an offspring but also to a decrease in milk yield, a decrease in the breeding value and in some cases infertility. Brucellosis (20-33.7%) was the first agent to be seen in the investigations of the infections causing abortion in sheep and this was followed by campylobacteriosis, chlamydiosis, listeriosis, and salmonellosis [3,19,20]. Campylobacteriosis is widely occurred all over the world and can be transmitted to people in contact with food, water, livestock and domestic animals, especially poultry [21,22]. Campylobacteriosis is the important cause of abortion in the sheep in many countries including Turkey [23-27]. Yardımcı et al.[28], reported that blood sera samples taken from sheep in Van region were analyzed by ELISA and detected *Campylobacter* antibody positivity in 39% of samples.

Many studies have been conducted to show *Campylobacter* spp. existence in sheep in many parts of the world. In the USA, Hansen et al.[29], reported as 5-17% risk of abortion due to *Campylobacter* species. Fallah et al.[25], have investigated 132 aborted sheep fetuses by PCR and showed 12 (9.09%) *C. fetus* subsp. *fetus* and 2 (1.51%) *C. jejuni* in Iran. Allsup [30], reported that Campylobacteriosis was the third responsible agent in sheep abortion and

increased from 6.8% in 1982 to 13.1% in 1984 in England. Species were determined according to the order of prevalence as *C. jejuni*, *C. fetus* subsp. *fetus* and *C. coli*.

Campylobacter species cause serious problems for animal and human health in our country as well as in the world and cause labor and economic losses. Karaman and Küçükkayan [31], have reported that *Campylobacter* spp. were isolated in 4 (1.3%) out of 297 aborted lambs obtained from different provinces between 1993-1997. In a similar study conducted by Küçükayan et al.[6], *Campylobacter* spp. were isolated in 6 (1.29%) out of 463 fetuses in 2003-2007 and all of them were identified as *C. fetus* subsp. *fetus*. Diker [32], had isolated *C. fetus* subsp. *fetus* from 15 (12,09%) out of 124 aborted sheep fetuses. Kenar et al.[33], reported that they isolated *Campylobacter* spp. in 20 (6,6%) of 303 aborted sheep fetuses. Kenar and Erganis [34], investigated 35 aborted sheep fetuses in Samsun and neighboring provinces during lambing season in 1991-1992 and *Campylobacter* spp. were isolated in 8 (22.9%) samples of which 5 (62.5%) were *C. fetus* subsp. *fetus*, 2 (25%) were *C. jejuni* and 1 (12.5%) was aerotolerant *Campylobacter*. Ekin et al.[24], investigated the presence of *Campylobacter* spp. in the gallbladder of healthy sheep in 2000 and 2002 years in Van region and found the *Campylobacter* spp. year-based prevalence as 27 (24.6%) and 24 (21.8%), respectively. Of the 27 *Campylobacter* strains isolated in 2000, 14 were identified as *C. jejuni*, 7 as *C. fetus*, 3 as *C. coli* and 3 as *C. lari*. Yeşilmen [12], have isolated the *Campylobacter* spp. in 10 (10%) out of 100 aborted sheep fetus in Diyarbakır province. Seven (70%) of the isolates were identified as *C. fetus* subsp. *fetus* and 3 (30%) were determined as *C. jejuni*. Büyük et al.[2], isolated *Campylobacter* spp. from 4 (10.25%) of 39 vaginal swab samples taken from sheep in Kars region. In a study conducted by Karakus [35], in Kars region, while both cattle and sheep have an important role as a source of *C. jejuni*, it was found that sheep played a more important role especially in the spread of *C. coli* to the environment.

In the present study, vaginal swab samples collected from sheep herds with abortion were examined in terms of *Campylobacter* species. *Campylobacter* spp. isolation was achieved in 8 (2.28%) vaginal swab samples. As the result of species-specific PCR analysis of isolates, 5 (62.5%) were identified as *C. coli* and 3 (37.5%) were *C. jejuni*. *Campylobacter* spp. isolation rate has varied between 1.2% and 92% in sheep in the world and in Turkey [2,26,36,37]. The results of this study were consistent with lots of researches. It is suggested that the factors cause the differences among the studies are the transport conditions of samples to laboratory, age and number of sampled animals, sampling season, isolation method and selective media used, hygiene and geographic structure [8,38].

In this study, it was revealed that *Campylobacter* infections should be taken into consideration in abortion cases occurring in sheep. It is also important since sheep can contaminate the environment and food with secreting the *C. jejuni* and *C. coli* and may play important role in human beings. Increased rate of isolation of *C. coli* from sheep will need more epidemiological investigations on this species as the *C. jejuni* is the primarily thermophilic agent in abortion cases.

REFERENCES

1. Türkiye İstatistik Kurumu: Hayvansal Üretim İstatistikleri 2017. http://www.tuik.gov.tr/PreTablo.do?alt_id=1002; *Accessed:* 30.05.2018.

2. Büyük F, Çelebi Ö, Şahin M, Ünver A, Tazegül E: *Brucella* and *Campylobacter* mixed infection in two different sheep and goat herds. *Kafkas Univ Vet Fak Derg*, 17 (Suppl. A): S177-S180, 2011. DOI: 10.9775/kvfd.2010.3134

3. Gürtürk K, Solmaz H, Ekin İH, Aksakal A, Gülhan T: Bacteriological and serological examinations of aborting sheep in Van region. *YYÜ Vet Fak Derg*, 11 (2): 19-22, 2000.

4. Diker S: *Campylobacter*, *Arcobacter* ve *Helicobacter* infeksiyonları. **In**, Aydın N, Paracıklıoğlu J (Eds): Veteriner Mikrobiyoloji. 237- 249, İlke Emek Yayınları, Ankara, 2006.

5. Fiorentino MA, Stazionati M, Hecker Y, Morsella C, Cantón G, Harry HR, Velilla AV, Vaulet LG, Fermepin MR, Bedotti DO: *Campylobacter fetus* subsp. *fetus* ovine abortion outbreak in Argentina. *Rev Electron Vet*, 18, 1-11, 2017.

6. Küçükayan U, Dakman A, Ülker U, Müştak K: Investigation of sheep sera and foetuses for the identification of abortifacient bacterial agents. *Etlik Vet Microbiol Derg*, 18, 11-16, 2007.

7. Horrocks SM, Anderson RC, Nisbet DJ, Ricke SC: Incidence and ecology of *Campylobacter jejuni* and *coli* in animals. *Anaerobe*, 15, 18-25, 2009. DOI: 10.1016/j.anaerobe.2008.09.001

8. Stanley K, Jones K: Cattle and sheep farms as reservoirs of *Campylobacter*. *J Appl Microbiol*, 94, 104-113, 2003. DOI: 10.1046/j.1365-2672.94.s1.12.x

9. Rizzo H, Gregory L, Beraldi F, Carvalho FA, Pinheiro ES: *Campylobacter* isolation from the feces of sheep with a history of reproductive disorders bred in the of Sao Paulo, Brazil. *Semin Cienc Agrar*, 36 (6): 4207-4214, 2015. DOI: 10.5433/1679-0359.2015v36n6Supl2p4207

10. Roug A, Byrne BA, Conrad PA, Miller WA: Zoonotic fecal pathogens and antimicrobial resistance in county fair animals. *Comp Immunol Microbiol Infect Dis*, 36 (3): 303-308, 2013. DOI: 10.1016/j.cimid.2012.11.006

11. Pao S, Hagens BE, Kim C, Wildeus S, Ettinger MR, Wilson MD, Watts BD, Whitley NC, Porto-Fett ACS, Schwarz JG, Kaseloo P, Ren S, Long III W, Li H, Luchansky JB: Prevalence and molecular analyses of *Campylobacter jejuni* and *Salmonella* spp. in co-grazing small ruminants and wild-living birds. *Livest Sci*, 160, 163-171, 2014. DOI: 10.1016/j.livsci.2013.11.020

12. Yeşilmen S, Gül K: Isolation, identification and antibiotic susceptibility of *Campylobacter* spp. in aborted sheep fetuses. *Med Weter*, 63 (10): 1184-1186, 2007.

13. Skirrow MB: Diseases due to *Campylobacter*, *Helicobacter* and related bacteria. *J Comp Pathol*, 111 (2): 113-149, 1994. DOI: 10.1016/S0021-9975(05)80046-5

14. Skirrow MB, Benjamin J: '1001' Campylobacters: Cultural characteristics of intestinal Campylobacters from man and animals. *J Hyg (Lond)*, 85, 427-442, 1980.

15. Vandamme P, Goossens H: Taxonomy of *Campylobacter*, *Arcobacter* and *Helicobacter*: A review. *Zentralbl Bakteriol*, 276, 447-472, 1992. DOI: 10.1016/S0934-8840(11)80671-7

16. Sambrook J, Russell D: Molecular Cloning: A Laboratory Manual. 3rd ed., Cold Spring Harbor Laboratory Press, New York. 2001.

17. Wang G, Clark CG, Taylor TM, Pucknell C, Barton C, Price L, Woodward DL, Rodgers FG: Colony multiplex PCR assay for identification and differentiation of *Campylobacter jejuni*, *C. coli*, *C. lari*, *C. upsaliensis*, and *C. fetus* subsp. *fetus*. *J Clin Microbiol*, 40 (12): 4744-4747, 2002. DOI:

10.1128/JCM.40.12.4744-4747.2002

18. Iraola G, Hernandez M, Calleros L, Paolicchi F, Silveyra S, Velilla A, Carretto L, Rodríguez E, Pérez R: Application of a multiplex PCR assay for *Campylobacter fetus* detection and subspecies differentiation in uncultured samples of aborted bovine fetuses. *J Vet Sci,* 13 (4): 371-376, 2012. DOI: 10.4142/jvs.2012.13.4.371

19. Muz A, Ertaş HB, Öngör H, Gülcü HB: Bacteriologic, serologic and pathologic studies on abortus cases of goats and sheep in Elazığ and it's vicinity. *Turk J Vet Anim Sci*, 23, 177-188, 1999.

20. Zhang H, Song S, Wang B, Jiang Y, Wu W, Guo F, Liu Y, Wang Q, Zhang J, Zhang H, Sheng J, Wang Y, Chen C: *Brucella melitensis* isolated from aborted cow and sheep fetuses in Northwest of China. *Kafkas Univ Vet Fak Derg*, 24 (2): 307-310, 2018. DOI: 10.9775/kvfd.2017.18881

21. Aslantaş Ö: Isolation and molecular characterization of thermophilic *Campylobacter* spp. in dogs and cats. *Kafkas Univ Vet Fak Derg*, 25 (3): 341-348, 2019. DOI: 10.9775/kvfd.2018.20952

22. Issa G, Basaran Kahraman B, Adıguzel MC, Yılmaz Eker F, Akkaya E, Bayrakal GM, Koluman A, Kahraman T: Prevalence and antimicrobial resistance of thermophilic *Campylobacter* isolates from raw chicken meats. *Kafkas Univ Vet Fak Derg*, 24 (5): 701-707, 2018. DOI: 10.9775/kvfd.2018.19741

23. Salihu MD, Junaidu AU, Oboegblem SI, Egwu GO: Prevalence and biotypes of *Campylobacter* species isolated from sheep in Sokoto state, Nigeria. *Int J Anim Vet Adv*, 1 (1): 6-9, 2009.

24. Ekin IH, Gürtürk K, Arslan A, Boynukara B: Prevalence and characteristics of *Campylobacter* species isolated from gallbladder of slaughtered sheep in Van, (Eastern) Turkey. *Acta Vet Brno*, 75, 145-149, 2006. DOI: 10.2754/avb200675010145

25. Fallah S, Hamali H, Joozani RJ, Zare P, Noorsaadat G: A molecular (PCR) survey on abortions caused by *Campylobacter* spp. in sheep flocks located on the suburb of Tabriz. *IJVST*, 6 (1): 23-29, 2014.

26. Ertaş HB, Ozbey G, Kılıç A, Muz A: Isolation of *Campylobacter jejuni* and *Campylobacter coli* from the gall bladder samples of sheep and identification by polymerase chain reaction. *J Vet Med B,* 50 (6): 294-297, 2003. DOI: 10.1046/j.1439-0450.2003.00678.x

27. Wu Z, Sippy R, Sahin A, Plummer A, Vidal A, Newell D, Zhanga Q: Genetic diversity and antimicrobial susceptibility of *Campylobacter jejuni* isolates associated with sheep abortion in the United States and Great Britain. *J Clin Microbiol*, 52 (6): 1853-1861, 2014. DOI: 10.1128/JCM.00355-14

28. Yardımcı H, Boynukara B, Akan M, Diker KS: Use of ELISA for detection of *Campylobacter* antibodies in sheep district of Van. *YYÜ Vet Fak Derg*, 9 (1-2): 5-8, 1998.

29. Hansen DE, Hedstrom OR, Sonn RJ, Synder PS: Efficacy of a vaccine to prevent *Chlamydia* or *Campylobacter* induced abortions in ewes. *J Am Vet Med Assoc*, 196 (5): 731-734, 1990.

30. Allsup TN: Ovine *Campylobacter* Abortion, Luxembourg, Ccommission of the Europen Communities, 93-107, 1985.

31. Karaman Z, Küçükayan U: 1993-1997 yılları içinde enstitümüze gönderilen atık yapan koyun kan serumları ve materyallerinin serolojik ve mikrobiyolojik yoklama sonuçları. *Etlik Vet Mikrobiyol Derg*, 11 (1-2): 2000.

32. Diker KS: Studies on the identification of *Campylobacter* species isolated from sheep and cattle. *Doğa Bilim Derg*, 9, 232-240, 1985.

33. Kenar B, Erganiş O, Kaya O, Güler L: Bacteriological and serological survey on *Brucella*, *Campylobacter*, *Salmonella* and *Chlamydia* infections caused to sheep abortion in Konya region (central Anatolia) in Turkey. *Veterinarium*, 1, 17-20, 1990.

34. Kenar B, Erganiş O: Isolation and antibiotic susceptibility of *Campylobacter* spp. in aborted ovine fetuses in the central Black Sea. *Veterinarium*, 5, 4-11, 1990.

35. Karakuş S: Thermophilic *Campylobacters* isolation, identification and molecular typing from cattle, sheep and humans in Kars area. *PhD Thesis*, Kafkas University, Institute of Health Sciences, Turkey, 2011.

36. Açık MN, Çetinkaya B: Heterogeneity of *Campylobacter jejuni* and *Campylobacter coli* strains from healthy sheep. *Vet Microbiol*, 115 (4): 370–375, 2006. DOI: 10.1016/j.vetmic.2006.02.014

37. Hamali H, Fallah S, Joozani RJ, Zare P, Noorsaadat G: Detection of *Campylobacter* spp. in sheep aborted fetuses by PCR. *Trends Life Sci*, 3 (2): 49-56, 2014.

38. Sanad YM, Jung K, Kashom I, Zhang X, Kassem II, Saif YM, Rajashekara G: Insights into potential pathogenesis mechanisms associated with *Campylobacter jejuni*-induced abortion in ewes. *BMC Vet Res,* 10:274, 2014. DOI: 10.1186/s12917-014-0274-8