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Detection of *Campylobacter fetus* in aborted ewes in Sulaimani province by PCR

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Abstract

Abortion is one of the most critical factors affecting lambing rates and, as a result, sheep farm profitability. It is also significant from a zoonotic viewpoint, in addition to financial losses. In sheep flocks, *Campylobacter fetus* causes infectious infertility, embryonic death, and miscarriages. The study investigated *C. fetus* from aborted fetuses and vaginal swab samples collected from sheep flocks in the Sulaimani province by the polymerase chain reaction. Thirty-eight aborted fetuses and 70 vaginal swabs were collected from sheep flocks in three districts of Sulaimani province (Kalar, Said Sadiq, and Chamchamal) from March 2018 to June 2019. The pathogen was identified in clinical specimens using conventional PCR. *C. fetus* was isolated in 16 of 38 aborted fetuses 42.1% and 13 of 70 vaginal swabs from aborted ewes 18.6%. The *C. fetus* gene 16S rRNA was sequenced and received the accession number MW694741 in NCBI GenBank. Phylogenetic analysis of 16S rRNA gene sequences designated that the *C. fetus* isolates formed a separate branch displayed the highest similarity and clustered with MN203686.1 and EU773268.1 accessions in a specific clade. A lower degree of affinity of *C. fetus* was revealed with *Campylobacter coli* and *Campylobacter jejuni*.

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Introduction

Abortion is one of the most significant matters in sheep breeding, and it is financially damaging to the farmer (1,2). In economies where lamb is the primary source of animal protein, ovine abortion is essential (3,4). Different bacterial, viral, and protozoal diseases have been associated with infectious ovine abortions. These infections are critical in terms of public health and the economy. Brucellosis, campylobacteriosis, chlamydiosis, and salmonellosis are the most common bacterial infections that cause abortion (5,6). *Campylobacter* was secluded from aborted sheep fetuses in 1909, and the name *Campylobacter* was given to it in 1963. These organisms cause abortion in cows and ewes, as well as severe enterocolitis in humans. *C. fetus subsp. fetus* or *Campylobacter jejun* are the source of this disease. Both organisms are capable of causing abortion epidemics indicated by substantial lesions in the placenta, fetal tissues, or both (7-9). *Campylobacter jejuni*, *C. coli*, and *C. fetus subsp. fetus* is common in the world and causes reproductive diseases in sheep. They are Gram-negative, motile and microaerophilic. Environmental samples such as soil, water, and food can be contaminated with *Campylobacter* species resulting from contact with contaminants such as feces and aborted fetuses (10,11). Abortion, stillbirths, and the birth of weak lambs are some of the clinical signs of campylobacteriosis in sheep (12-14). This disease spreads in flocks by introducing new carrier animals, and pregnant ewes could become infected by drinking contaminated water or ingesting contaminated feed (15-17). *Campylobacter fetus* is now considered a zoonotic disease. Human infections are considered to be introduced through cattle and sheep products. Human infection with *C. fetus* usually begins with the bacteria being consumed orally, followed by colonization of the intestine. Some colonized individuals induce diarrhea. Occasionally, *C. fetus* causes severe systemic infections (18-

20). Rapid diagnosis of an agent causing abortion is of great importance in preventing and controlling the disease. Old diagnostic methods of campylobacteriosis are time-consuming, somewhat challenging, and are not always accurate. Thus, molecular methods like PCR have been welcomed in recent years, particularly in research studies (21,22). This study aimed to investigate *C. fetus*, one of the critical abortion agents, from aborted sheep fetuses and vaginal swab samples collected from sheep flocks in the Sulaimani province by PCR.

Materials and methods

Ethical approval

The study was carried out in the Research Center, College of Veterinary Medicine, University of Sulaimani, from March 2018 - June 2019 and approved by the Ethical Committee (Approval No. 01268/20Feb2018).

Study area and sample collection

Between March 2018 and June 2019, 108 samples were obtained from various flocks in three districts of Sulaimani province with a history of abortion. We collected 38 samples of aborted fetuses. Eighteen samples had been from Kalar, 13 from Said Sadiq, and seven from Chamchamal. In addition, 70 vaginal swab samples had been taken from the vaginas of aborted ewes, 38 from Kalar, 12 from Said Sadiq, and 20 from Chamchamal. In these districts, the sheep flocks management method is traditional; different people own the animal flocks. Indoors, the sheep are fed grain, hay, and silage before being released to graze on pasture. Various animal species might graze on the same pasture, or flocks might share rams to enhance fertility. Tissue from recently aborted fetuses (liver and spleen) and their dams (vaginal swabs) with abortions within the previous 2–4 days was obtained using disposable blades and scissors. Collected samples were placed in plastic containers, labeled, and sent in a refrigerated box to the Research Center of the College of Veterinary Medicine / University of Sulaimani, where they were identified as *C. fetus* the same day.

Extraction of DNA

A DNA extraction kit was used on the samples to extract the DNA (GeNet Bio, South Korea). Following the manufacturer's instructions, the process was carried out. DNA quality was measured spectrophotometrically, and low concentration samples (lower than 100 ng/µL) were eliminated from further analyses (23).

Oligonucleotides and PCR amplification

Hossein *et al*. (24) presented the primers used for amplifying a 265 bp segment of the 16S rRNA gene, with forward (5´-TTTGTTAGGGAAGAACCATG-3') and reverse (5´-CGCAATGGGTATTCCTGGT-3') primers. Macrogen® (South Korea) progressed the primers for our research. The total DNA was amplified using PCR Add Start Taq Master (PCR Add Start Taq Master) (Korea, Add bio). 0.2 mL PCR tubes were used for the experiment. The PCR tube included l0 µL of master mix, 5 µL of DNA, and l µL (10 pmol) of each forward and reversed primer. The ultimate volume of 20 µL was achieved by adding 3 µL of DEPC-treated water (25,26). The thermal cycler method began with an initial denaturation at 95 °C for five minutes. The samples were then subjected to 30 cycles of denaturation (95°C for 1 minute), annealing (55°C for 1 minute), and extension (72°C for 1 minute), a five-minute final extension at 72°C was also included. The PCR products were examined after loading 7 µL on a 1 % agarose gel in 1× Tris/Borate/EDTA (TBE) buffer (27,28). A 5 µL safe dye was used to stain the gel. Using the Safe-Blue Illuminator/Electrophoresis System, electrophoresis was performed for 50 minutes at 120 volts. By comparing PCR result amplicons to a 100 bp DNA ladder, migration patterns were studied (29,30).

Sequencing of the 16S rRNA gene and phylogenetic analysis

South Korea's Macrogen Sequencing Facility sequenced the PCR results of the *C. fetus* 16S rRNA gene. After being sequenced many times, the gene sequences were submitted to GenBank to authenticate each nucleotide's identification to get accession numbers. Prokaryotes were identified and classified using a sequencing study of the small subunit ribosomal RNA (16S rRNA) gene. Microbial diversity is also estimated using the sequence of 16S rRNA genes amplified from the environment. The pathogen causing intestinal disease and abortions in sheep, *C. fetus*, was isolated from northern Iraq in 2018. The partial 16S rRNA gene sequence (265 bp) of the strain was collected from the vaginal swabs of the aborted ewes, and the two strands were sequenced. The sequence was compared with those in available databases using BLAST and aligned with its nearest neighbors using Mega X (31-33).

Results

Samples

Genomic DNA was successfully extracted from fetal samples and vaginal swabs of aborted ewes. In the current investigation, 108 samples were taken from Kalar, Said Sadiq, and Chamchamal, where abortions had been observed. Sixteen samples (42.1%) from aborted fetuses and 13 samples (18.6 %) from vaginal swabs of PCR were used to identify *C. fetuses* in aborted ewes (Table 1).

Identification of *Campylobacter fetus*

According to agarose gel electrophoresis, the *campylobacter fetus* was positive for the 16S rRNA gene in the present study, which indicated a 265 bp amplicon (Figure 1). The sequencing of the PCR product was determined to

corroborate the results, which was given the NCBI GenBank accession number MW694741 and given the name *C. fetus.*

Table 1: PCR results for the detection of *Campylobacter fetus* in aborted fetuses and vaginal swabs of aborted ewes

District name	Fetal samples		Vaginal swabs	
	Samples tested	Samples positive (No. %)	Samples tested	Samples positive (No. %)
Kalar	18	9 (50.0)	38	7 (18.4)
Said Sadiq	13	4 (30.8)	12	2 (16.7)
Chamchamal	7	3 (42.9)	20	4 (20.0)
Total	38	16 (42.1)	70	13 (18.6)

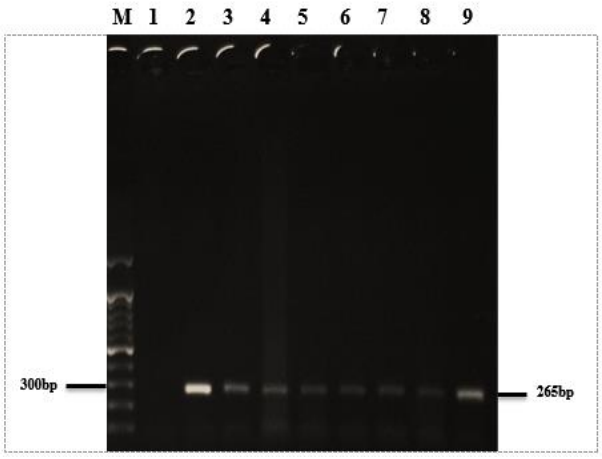

Figure 1. Specific amplification of target DNA from *Campylobacter* by PCR using specific primers. Lane M: Show 100 bp DNA size marker, lane 1: Negative control (no DNA in the PCR reaction mix), lane 2-9: An aborted ewe's samples (265bp).

Phylogenetic analysis

The taxonomic position of *C. fetus* as revealed by neighbor-joining analysis of the 16S rRNA gene sequence alignment (Figure 2). All accessions were clustered into three main classes. 16S rRNA gene sequence phylogenetic analysis showed that the *C. fetus* isolates formed a separate branch, displayed the highest similarity, and clustered with uncultured *Campylobacter* species (Accession number MN203686.1) uncultured bacterium BHSD (Accession number EU773268.1) in a specific clade. *C. fetus* has a reduced affinity for the *Campylobacter coli* and *Campylobacter jejuni* bacteria.

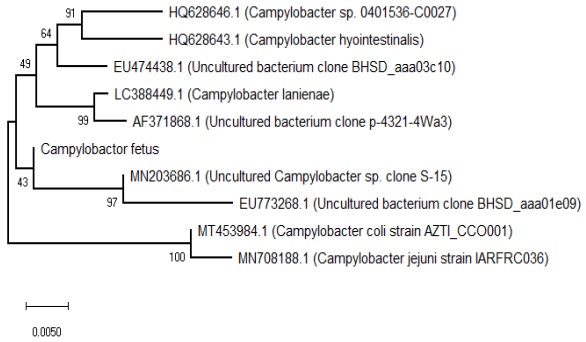

Figure 2. A maximum-likelihood dendrogram based on 16S rRNA gene sequences revealed the phylogenetic relations between *C. fetus* and some similar taxa. In branch nodes, bootstrap values based on 100 replicates are presented. Bar = 0.005 substitutions per nucleotide position.

Discussion

Abortions caused by infectious agents in sheep breeding are a fundamental problem. These agents lead to significant economic losses, a loss of offspring, a decrease in milk yield, a decrease in breeding value, and infertility (34). In many places, campylobacteriosis is a significant cause of sheep abortion. The majority of abortions occur in the final month of pregnancy. Infection causes include the placenta, the fetus, the birth fluids, the vaginal discharge, and the feces of the ewe (35). In the present study, vaginal swabs from aborted ewes and aborted fetuses were investigated by PCR to detect *Campylobacter*. Many studies previously used this method (24,36).

According to our data, in sheep, campylobacteriosis causes abortion. Moreover, *C. fetus* is the most common cause of ewe abortion in Sulaimani province. *C. fetus* was isolated from 42.1% of aborted fetuses and 18.6% of vaginal swabs from aborted ewes using PCR. Compared to traditional bacteriological tools, rapid identification of the causal agent utilizing molecular techniques shows the effectiveness of PCR techniques as a practical alternative to laboratory diagnosis. This is especially significant when it comes to detecting fastidious microorganisms like the *Campylobacter* genus (37). Detection rates in the present study are considerably higher than published research on abortion in sheep with infection of *C. fetus* from other countries such as Iran 7.7% (24) and Turkey 7% (38). Differences between studies may be attributed to geographical region, diagnostic techniques, the animal's breed, collection, and timing of materials. On the other hand, our research results contradict those of other studies (7,39), which detected that *C. jejuni* and *C. coli* were the most

prevalent *campylobacter* species in sheep diseases and abortion in the United States and Turkey, respectively.

The current study recorded *C. fetus* isolated from aborted ewes for the first time in Iraq, and it was registered in the GenBank database (Ac.: MW694741). Focused on the 16S rRNA gene, all accessions were clustered into three main classes: the *C. fetus* isolates. The present study formed a separate branch and showed the highest similarity with uncultured *Campylobacter* spp. (Accession number MN203686.1) and uncultured bacterium BHSD (Accession number EU773268.1). Both MN203686.1 and EU773268.1 were isolated from the same animal (sheep) but in different countries. According to Mohakud *et al.* (40) and Ley *et al*. (41), the closely related isolates MN203686.1 and EU773268.1 were isolated in the feces of sheep. Regarding these findings, the present study suggests further future studies to detect a genetic relation between *C. fetuses* isolated from the vagina and those isolated from sheep feces. Because of limited genetic information on *C. fetus* in those states, future studies should focus on whole-genome sequencing of all *Campylobacter* strains in Iraq and surrounding countries.

Conclusion

Campylobacteriosis is a significant cause of abortion of ewes in Sulaimani province, accounting for the majority of sheep abortions in the area. *C. fetus* is the most pathogenic *Campylobacter* species in our region, causing 42.1 % of ewe abortions.

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Conflict of interest

The author declares that he has no conflict of interest.

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الكشف عن العطيفة الجنينية في النعاج المجهضة في محافظة السليمانية بوساطة تفاعل البلمرة المتسلسل

ايمان ظاهر عارف

فرع الاحياء المجهرية، كلية الطب البيطري، جامعة السليمانية، السليمانية، العراق

الخلاصة

يعتبر الإجهاض من أهم العوامل التي تؤثر على معدلات الحمل، وناتج ذلك على ربحية حقول الأغنام. كما أنها مهمة من وجهة نظر انها ذات منشأ حيواني. بالإضافة إلى الخسائر المالية. في قطعان الماشية والأغنام، تسبب العطيفة الجنينية عقم معدي، موت جنيني وإجهاضات. تم التحري عن العطيفة الجنينية في أجنة النعاج المجهضة وعينات المسح المهبلية التي تم جمعها من قطعان الأغنام في محافظة السليمانية بوساطة تفاعل البلمرة المتسلسل. جمع ٣٨ جنينا مجهضا و ٧٠ مسحة مهبلية من النعاج في ثلاث اقضية من محافظة السليمانية (كلار، سيد صادق وجمجمال) من اذار ٢٠١٨ إلى حزيران ٢٠١٩. استخدم تفاعل البلمرة المتسلسل لتحديد المسبب المرضي. تم عزل العطيفة الجنينية في ١٦ من ٣٨ من الأجنة المجهضة بنسبة ٤٢,١٪ و ١٣ من ٧٠ مسحة مهبلية من النعاج المجهضة بنسبة ١٨,٦٪. تم تحديد التسلسل الجيني للحامض النووي الرايبوزي نوع ١٦S للعطيفة الجنينية وحصل على رقم الانضمام MW694741 في بنك جينات ان سي بي اي. حدد التحليل الوراثي لتسلسل الجيني لحامض النووي الرايبوزي نوع ١٦S إلى أن العطيفة الجنينية المعزولة شكلت فرع منفصل وعرض أعلى تشابه وتم تجميعها مع مدخلات MN203686.1, EU773268.1 في شفرة محددة. كانت اقل درجة تقارب لجرثومة العطيفة الجنينية مع العطيفة القولونية والعطيفة الصائمية.