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Investigation of *Mycoplasma bovis* and *Ureaplasma diversum* from Bovine Aborted Fetuses in Northeast Anatolia Region by PCR

Ediz Kağan ÖZGEN[1], Berna YANMAZ[1]✉, Mustafa ULUCAN[1], Perihan Şerifoğlu BAĞATIR[1], Murat ÖZMEN[1], Elif Karadeniz PÜTÜR[1]

[1]Erzurum Veterinary Control Institute, Erzurum/TURKEY

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Abstract: Abortion is one of the most important problems in animal husbandry due to its hindrance in obtaining offspring, labor, and economic losses. Although *Mycoplasma bovis (M. bovis)* and *Ureaplasma diversum* (*U. diversum*) are the bacteria found in the genital flora, they are considered as important pathogens that cause bacterial abortion. The aim of this study was to investigate the presence of *M. bovis* and *U. diversum* in aborted cattle fetuses from Northeastern Anatolia province by PCR method. In this study, a total of 187 cattle fetuses samples (lung and liver tissues and abomasum content of fetuses) were examined from March to June 2019. The fetuses samples were taken from the provinces of Ağrı, Ardahan, Artvin, Erzincan, Erzurum, Gümüşhane, Iğdır, and Kars. *M. bovis* was positive in 4.8% (9/187) and *U. diversum* was positive in 2.7% (5/187) of the fetus samples. As a result, both agents should be considered in routine investigations when investigating the infectious causes of abortion cases. Further studies may be beneficial to determine the pathogenicity and type strains of *M. bovis* and *U. diversum* in aborted cattle fetuses.

Keywords: Abortion, Cattle, *Mycoplasma bovis*, *Ureaplasma diversum*

Kuzeydoğu Anadolu Bölgesinde Sığır Aborte Fötuslarından *Mycoplasma bovis* ve *Ureaplasma diversum*'un PCR ile Araştırılması

Özet: Abort vakaları iş gücü, yavru kaybedilmesi ve ekonomik kayıplardan dolayı sığır yetiştiriciliğindeki en önemli problemlerden biridir. *Mycoplasma bovis (M. bovis)* ve *Ureaplasma diversum (U. diversum)* etkenleri genital florada bulunan bakteriler olsalar da bakteriyel aborta neden olan etkenler arasında yeralan önemli patojenler olarak kabul edilmektedir. Bu çalışmanın amacı Kuzeydoğu Anadolu illerinden alınan aborte sığır fötuslarında abort nedenleri arasında yeralan *M. bovis* ve *U. diversum*'un varlığının PCR metodu ile araştırmaktır. Bu çalışmada Mart-Haziran 2019 tarihleri arasında toplam 187 büyükbaş fetüs örneği (akciğer ve karaciğer dokuları ve fetusların abomasum içeriği) incelenmiştir. Fetüs örnekleri Ağrı, Ardahan, Artvin, Erzincan, Erzurum, Gümüşhane, Iğdır ve Kars illerinden alındı. İncelemesi yapılan fötusların %4,8 (9/187)'inde *M. bovis*, %2,7 (5/187)'sinde *U. diversum* pozitif tespit edildi. Araştırma sonucunda abort vakalarının enfeksiyöz nedenleri araştırılırken her iki etkenin de rutin incelemelerde dikkate alınması gerektiği düşünülmektedir. Daha ileri çalışmalar, aborte sığır fetüslerinde *M.* bovis ve *U. diversum*'un patojenitesini ve suş tipini belirlemek için yararlı olabilir.

Anahtar Kelimeler: Abort, Sığır, *Mycoplasma bovis*, *Ureaplasma diversum*

1.Introduction

Abortion is one of the most important problems in animal husbandry due to its hindrance in obtaining offspring, labor, and economic losses. The causes of abortion in cattle are mainly due to infectious agents (1). *Mycoplasma bovis (M. bovis)* and *Ureaplasma diversum (U. diversum)* are the bacteria found in the genital flora and accepted as important pathogens among bacterial abortion agents (2, 3). Even though *M. bovis* is mainly associated with bronchopneumonia in dairy cattle, it may also cause mastitis, arthritis, otitis, vulvovaginitis, infertility, endometritis, and dystocia (3, 4). *Mycoplasma* species are reported to be responsible for 4.4% of bovine abortions (5). Although the *U. diversum* and *M. bovis* belong to the same family, *Ureaplasma* species may easily distinguish from

✉: bernayanmaz1@gmail.com

mycoplasmas by their ability to hydrolyze urea (6). *Ureaplasmas* are generally considered as opportunistic pathogens (7), and the semen of the carrier bull is transmitted to healthy female cattle that may cause vulvovaginitis, infertility and abortion (6, 8).

Abortions caused by *U. diversum* usually occurs in the third trimester of pregnancy (7). Various studies conducted in non-pregnant cattle revealed that 36-64% of cattle were infected with *U. diversum*. However, in studies conducted during the gestation period, a prevalence of 54-76% is reported in cattle. For isolation in bacteriological culture, it is necessary to have urea in the medium, but the culture processes are difficult because the products that result from the hydrolysis of the urea can damage the bacteria in the non-buffered media. To prevent this problem, therefore, PCR is used as a diagnostic method (9).

To the authors of knowledge, to the date, no report has investigated the prevalence of *U. diversum* and *M. bovis* in aborted fetuses in the Northeast Anatolia Region of Turkey. For this reason, this study was aimed to investigate the presence of *M. bovis* and *U. diversum* among abortion causes in aborted bovine fetuses by PCR method.

2.Materials and Methods

2.1.Samples

In this study, a total of 187 cattle fetuses were examined from March to June 2019. The samples were taken from the provinces of Ağrı, Ardahan, Artvin, Erzincan, Erzurum, Gümüşhane, Iğdır, and Kars.

Twenty-five mg of lung and liver tissues of aborted fetuses and 50 µl of abomasum contents were taken into 2 ml sterile homogenization tubes (GreenBeads, Roche). Samples were homogenized in tissue homogenization device (MagnaLyser, Roche) and 200 µl homogenate was taken into sterile 1.5 ml volume microcentrifuge tubes and stored at -20 ° C until DNA extraction.

2.2.DNA extraction

Commercial DNA extraction kit (Cador Pathogen Kit, Qiagen) was used for DNA extraction from homogenates obtained from samples of aborted fetus tissues and abomasum contents. DNA extraction was performed according to the kit protocol. The obtained DNA samples were stored at -20 ° C until PCR analysis.

3.Duplex PCR

16S rRNA gene-specific primers (Table 1) of bacteria were used to detect the presence of *M. bovis* and *U. diversum* in the DNA samples obtained (10).

The PCR assays were performed in a total volume of 25 µl with a reaction mix containing 2,5 µl of 10X reaction buffer, 20 pmol of each primer added, 200 µmol of deoxyribonucleotide triphosphate (dNTP) mix, and 0.5 µl of DNA polymerase. Two µl of extracted DNA was added to the reaction mix. The thermal cycling protocol included an initial denaturation of 95°C for 5 min, followed by 40 cycles of 94°C for 1 min, 50°C for 1 min and extension of 72°C for 1 min. This was followed by a final extension step at 72°C for 5 min.

Table 1: Primers used in PCR analysis

Agent	Target Gene	Primer 5'-3'	Size
M. bovis	16S rRNA	GTTTGATCCTGGCTCAGGAT	198 bp
		CAAACGCTTCCTTTTATATTAC	
U. diversum	16S rRNA	GTTTGATCCTGGCTCAGGAT	831 bp
		CTCATAAGCGAGCCGACATT	

Ethidium bromide was added to agarose gel to be used for electrophoresis. The PCR products were electrophoresed on 1,5 % (w/v) agarose gel. Products were visualized using an ultraviolet transilluminator. Bands of size 198 bp for *M. bovis* and 831 bp for U. diversum were considered positive.

3.Results

The samples of cattle aborted fetuses taken from 8 cities were examined by duplex PCR for *M. bovis* and *U. diversum*. *M. bovis* was positive in 4.8% (9/187) and *U. diversum* was found in 2.7% (5/187) of the examined fetus samples.

None of the samples taken from 7 cities except Erzurum were positive for *M. bovis* and *U. diversum* (Table 2).

Table 2: *M. bovis* and *U. diversum* PCR positivity by cities

City	*M. bovis* Positive	*U. diversum* Positive	Total Sample
Ağrı	0	0	27
Ardahan	0	0	8
Artvin	0	0	1
Erzurum	9	5	96
Erzincan	0	0	35
Gümüşhane	0	0	2
Iğdır	0	0	1
Kars	0	0	17
Total	9	5	187

Distribution of positivity according to sample districts is shown in Table 3. *M. bovis* positivity was not detected in 4 of 5 fetuses with *U. diversum*. *M. bovis* and *U. diversum* were both detected in a fetus sent only from Horasan district (Figure 1).

Table 3: Distribution of PCR positivity of *M. bovis* and *U. diversum* according to sampled Erzurum districts

District	*M. bovis*	*U. diversum*	Total Sample
Aşkale	2	1	19
Aziziye	1	0	5
Çat	0	1	2
Horasan	1	1	7
İspir	1	1	10
Köprüköy	1	0	10
Pasinler	1	0	15
Tortum	1	1	11
Yakutiye	1	0	12
Total	9	5	91

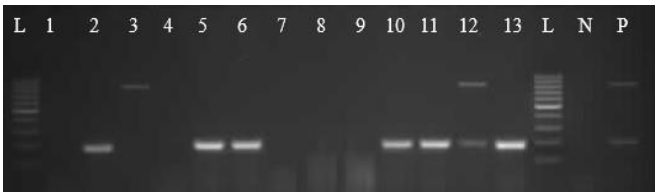

Figure 1: *M. bovis* and *U. diversum* PCR analysis. L: Ladder (100-1000 bp), N: Negative control, P: Positive control

4.Discussion

Abortion is the most important cause of economic losses in cattle breeding. Abortions are an important problem for breeders and countries by preventing the growth of herds around the world. In abortion cases, infectious causes generally occur at the herd level and many microorganisms have been reported to cause abortion (11). In this study, the presence of *M. bovis* and *U. diversum* in aborted bovine fetuses was determined by PCR method for the first time in our region.

M. bovis is an important bacterium that causes pneumonia, mastitis, arthritis, genital system diseases, and abortions, and *M. bovis* is diagnosed from aborted fetuses of domestic and wild cattle species (12). Although the investigation of M. bovis-related abortions is not common throughout the world, there are scientific studies in Germany, Ireland, and Australia (6). Abortion cases in cattle have been reported to occur during or after mastitis caused by *M. bovis. M. bovis* can be isolated from many organs of bovine aborted fetuses, but the best and a highly isolated sample of bacteria is the aborted fetus abomasal contents (13). In our country, researches have been conducted on other diseases such as pneumonia, mastitis caused by M. bovis (14). A study on the presence of *M. bovis* in cattle aborted fetuses has not been found in our country. It has been reported that abortion cases due to *M. bovis* are between 2-4% (15) and are consistent with the presented study findings. In the transmission of *M. bovis* to the genital canal is caused by contact with the sick and carrier animals in the herd or by providing pregnancy with contaminated sperm (16). In this case, if there are pneumonia and mastitis cases commonly caused by *M. bovis* and good herd management is not applied, pneumonia, mastitis, genital system diseases, and most importantly abortions will occur in other animals.

Tramuta et al. (10) developed a multiplex PCR method for the detection of bacterial, viral, and parasitic abortion agents in bovine abortions. In this study, 50 bovine aborted fetuses were examined and found 4% (2/50) *U. diversum* positive. Syrjala et al. (17) identified *U. diversum* in 13% of 93 aborted bovine fetuses between 1999 and 2006 in Finland. Trichard and Jacobs (18) found that Ureaplasma was positive in 8% of aborted bovine fetuses in their study in South Africa. As a result of the study, it was found that the rate of 2.7% in bovine abortion fetuses is consistent with previous studies.

Even though the culture method and PCR methods can be used for diagnosing both bacterial infections, however, the isolation of Ureaplasma species in bacteriological culture is difficult, laborious, and time-consuming. Moreover, faster results can be obtained with PCR methods (4, 7).

Cardoso et al. reported that the sensitivity and specificity of PCR were higher than culture in the diagnosis of *U. diversum*. Besides, bacteriological culture is reported to be insufficient for isolation of some serotypes of U. diversum (19).

In conclusion, *M. bovis* and *U. diversum* agents causing an abortion in fetus samples were analyzed by PCR method in

this study. Both agents should be considered in routine investigations when investigating the infectious causes of bovine abortion cases. Studies on the pathogenicity and typing of strains obtained in bovine abortion cases of *M. bovis* and *U. diversum*, which cause genital infections, infectious infertility and abortion in cattle, are considered to be beneficial.

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