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Short Communication

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Characterisation of Genital Mycoplasma Species from Preputial Swabs of Bucks and Rams [1]

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Abstract

Mycoplasma species are a major cause of mastitis, arthritis, pneumonia and reproductive disorders in small ruminants. Mycoplasmas in reproductive systems of males have been associated with diseases as orchitis, balanoposthitis and abnormal spermatozoa activity. The aim of this study was to detect *Mycoplasma* species that cause reproductive infections in bucks and rams. Total of 27 preputial swabs was collected from Saanen bucks and Kıvırcık rams at the Artificial Insemination (AI) Center of Uludag University in Turkey. Bacteriological culture methods, followed by Polymerase Chain Reaction (PCR) and Denaturing Gradient Gel Electrophoresis (DGGE) were used to detect and identify *Mycoplasma* species. The PCR-DGGE method identified one *M. bovigenitaliu*m and one *M. arginini* from two cases of orchitis in rams, and another *M. bovigenitalium* was identified from a buck with no clinical signs. The results showed that *Mycoplasma* species were present in the testicles of rams and bucks that were negative for Brucellosis, and likely causative organisms of orchitis which lead to reduced fertility.

Keywords: *Genital Mycoplasmas, PCR-DGGE, Preputial swab, Small ruminant*

Teke ve Koçların Prepusyal Svap Örneklerinden Genital Mikoplazma Türlerinin Karakterizasyonu

Öz

Mikoplazma türleri küçük ruminantlarda mastitis, artritis, pnömoni ve reprodüktif bozukluklara neden olabilmektedir. Erkek hayvanların reprodüktif sistemlerinde bulunan mikoplazmalar; orşitis, balanopostitis ve anormal spermatozoa aktivitesi gibi hastalıklarla ilişkilendirilmiştir. Bu çalışmada, teke ve koçlarda reprodüktif infeksiyonlara neden olan mikoplazma türlerinin tespiti ve karakterizasyonu amaçlandı. Uludağ Üniversitesi Suni Tohumlama Merkezi'nde bulunan Saanen ırkı tekelerden ve Kıvırcık ırkı koçlardan toplamda 27 adet prepusyal svap örnekleri toplandı. Mikoplazma türlerinin izolasyon ve identifikasyonu için bakteriyolojik kültür yöntemleri, Polimeraz Zincir Reaksiyonu (PCR) ve Denatüre Edici Gradient Jel Elektroforezi (DGGE) metodu uygulandı. PCR-DGGE metodu ile orşitis semptomu gösteren koçların birinden *M. bovigenitalium* ve diğerinden *M. arginini* identifiye edilirken; semptom göstermeyen bir tekeden ise *M. bovigenitalium* identifiye edildi. Elde edilen sonuçlar ile Brusellozis negatif olan koç ve tekelerin testislerinde, fertilite azalmasına yol açan orşitise neden olan Mikoplazma türlerinin varlığı ortaya konmuştur.

Anahtar sözcükler: *Genital Mikoplazmalar, PCR-DGGE, Prepusyal svap, Küçük ruminant*

INTRODUCTION

The reproductive disorders in caprines and ovines may be caused by some Mollicutes and *Mycoplasma* species [1]. *Mycoplasma* species, which are usually host specific, can infect man or animals causing clinical signs that include pneumonia, arthritis, infertility and abortion [2]. Some Mycoplasma diseases that affect small ruminants are listed

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by the OIE as they can cause major economic losses. These include contagious caprine pleuropneumonia, caused by *Mycoplasma capricolum* subsp. *capripneumoniae* in goats and contagious agalactia (CA) in sheep and goats, caused by *Mycoplasma agalactiae*, *Mycoplasma mycoides* subsp. *capri, Mycoplasma capricolum* subsp. *capricolum* and *Mycoplasma putrefaciens.* These CA infections have been associated with mastitis, arthritis, keratoconjunctivitis, abortion and atypical symptoms include respiratory and genital signs [3]. Other *Mycoplasma* species that infect small ruminants includes *Mycoplasma ovipneumoniae*, which is usually associated with respiratory disease; *Mycoplasma conjunctivae* that causes eye infections; and *M. arginini* which is thought to be a commensal, but may cause opportunistic infections. *Mycoplasma bovigenitalium*, previously called *Mycoplasma* ovine/caprine serogroup 11 [4], was originally isolated from infertile ewes having been introduced by infertile rams that showed abnormal sperm morphology and motility [5,6]. It has been associated with vulvovaginitis, epididymitis, orchitis, and infertility, resulting in economic losses.

The identification of different *Mycoplasma* species is technically difficult and expensive using conventional culture methods and mycoplasma species specific antiserum in growth inhibition tests [7]. Newer methods include specific polymerase chain reactions (PCR's) but these only identify one species unless they are multiplexed. More recently a microarray has been described but it is not widely used. The combined use of a PCR based on the 16S rDNA gene that will detect all *Mycoplasma* species, which can then be differentiated using denaturing gradient gel electrophoresis (DGGE) (PCR-DGGE) [8] to detect and identify the majority of *Mycoplasma* species is being used in a number of specialised laboratories. The aim of this study was to detect and identify mycoplasmas that cause reproductive infections by testing preputial swabs from bucks and rams.

MATERIAL and METHODS

Preputial swabs were collected from 17 bucks (Saanen) and 10 rams (Kivircik) kept at the Artificial Insemination (AI) Center of Uludag University in Turkey. The animals in semi-intensive system were mixed as bucks and rams and were kept together. All rams and bucks fed with a commercial concentrate diet with hay and water provided ad libitum. The animals were not vaccinated against CA and were clinically healthy, except for two rams which showed signs of orchitis in both testicles. Preputial swabs were placed into Stuart transport medium (cotton wrapped, Cultiplast, LP Italiana) and transported to the laboratory in cool containers at 4°C. All of samples were investigated for *Brucella* spp., *Mycoplasma* species and other bacterial infections.

Bacteriological Examination

All samples were inoculated into Mycoplasma Broth Base containing Mycoplasma Supplement G (Oxoid, UK), where ten-fold serial dilutions were made, and then plated onto Brucella selective agar (Oxoid, UK), Columbia blood agar with 5% sheep blood and MacConkey agar and Mycoplasma agar base containing Mycoplasma supplement G plates (Oxoid, UK). Columbia blood agar and MacConkey agar plates were incubated at 37°C for 24-48 h in an aerobic environment. In addition *Brucella* selective media were incubated 37°C in a 5-10% CO_2 atmosphere. Media were examined daily for up to seven days. All of the mycoplasma media were incubated at 37°C in a 5-10% CO_2 atmosphere and observed for three to seven days. The broth media were examined daily for signs of mycoplasma growth and the agar media were examined for mycoplasma-like colonies and typical 'fried-egg' colonies using a stereomicroscope. Suspect *Mycoplasma* colonies were tested for digitonin sensitivity and urease activity for Acholeplasmas and Ureaplasmas, respectively, using standard methods [7].

DNA Extraction and 16S rDNA PCR-DGGE

Genomic DNA was extracted directly from 1 mL of the broth cultures using the High Pure PCR Template Preparation Kit according to the manufacturer's instructions (Roche Diagnostics). The 16S rDNA gene PCR and DGGE were performed according to the method described by McAuliffe et al.[8] using GC341-F (universal) and R543 (mycoplasma specific) primers. Briefly, DGGE was performed using the Ingeny PhorU 2x2 apparatus (GRI Molecular Biology, Essex, UK). Samples (20 µL) were loaded on to 10% polyacrylamide/bis (37.5: 1) gels with denaturing gradients from 30 to 60% (where 100% is 7M urea and 40% (v/v) deionized formamide) in 1X TAE electrophoresis buffer. Electrophoresis was carried out at 100V at a temperature of 60°C for 18 h. Gels were stained with SYBR Gold (Cambridge BioScience, UK) in 1X TAE for 30 min at room temperature and visualized under UV illumination. The controls used included the type strains of: *M. agalactiae* (NCTC 10123), *M. bovigenitalium* (NCTC 10122), *M. mycoides* subsp. *capri* (NCTC F30), *M. arginini* (NCTC 10129), *M. ovipneumoniae* (NCTC 10151), *M. bovis* (NCTC 10131), *M. capricolum* subsp. *capricolum* (NCTC 10137) and *Ureaplasma diversum*.

RESULTS

Bacterial growth was observed in 3 (1 buck and 2 rams) out of 27 samples (11.1%) as indicated by turbidity in the broths and 'fried egg' colonies on mycoplasma plates. No bacterial colonies were detected on Columbia blood agar and Brucella selective media *(Table 1).* Digitonin sensitivity and urease activity tests were negative.

Three mycoplasma isolates were identified using the 16S rDNA PCR-DGGE method and compared to standard ruminant mycoplasma controls *(Fig. 1)*. Bacteriological examination and the 16S rRNA gene-based PCR-DGGE method identified two of the cultures as *M. bovigenitalium* and one as *M. arginini*. One of the *M. bovigenitalium* cultures and the *M. arginini* were isolated from cases of

Table 1. *Bacteriological and molecular findings obtained from preputial swabs from bucks and rams*

Host	No. of Sample	Number of *Mycoplasma* Species (positive samples) (%)	16S rDNA /DGGE Results (number of isolates)
Ram	10	2 (20%)	1 *(M. bovigenitalium)* 1 *(M. arginini)*
Buck	17	1 (5.9%)	1 *(M. bovigenitalium)*
Total	27	3 (11.1%)	3

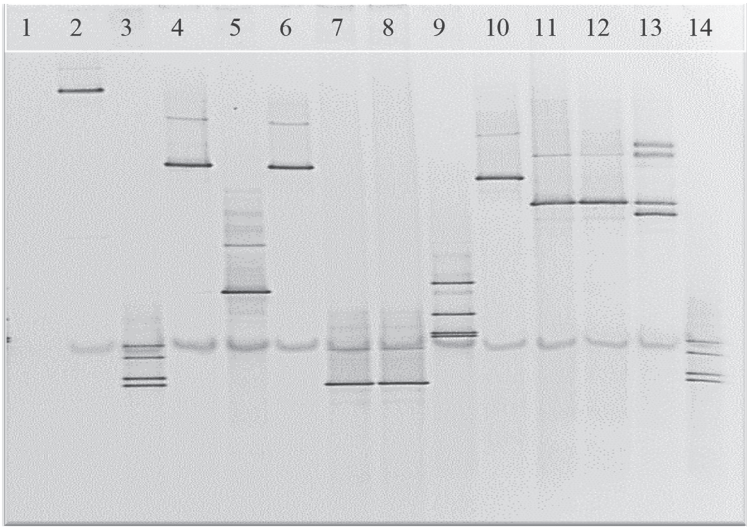

Fig 1. DGGE example gel of the amplified V3 region of 16 S rDNA gene. Lanes: 1, negative control; 2, *U. diversum* positive control; 3, *M. arginini* positive control; 4, *M. bovigenitalium* positive control; 5, *M.bovis* positive control; 6, *M.bovigenitalium* from example sample ; 7-8, *M. arginini* from example sample; 9, *M.agalactiae* positive control; 10, *M.ovipneumoniae* positive control; 11, *M.mycoides* subsp *capri* positive control; 12, unknown profile; 13, *M.capricolum* subsp *capricolum* positive control; 14, *M.arginini* 2nd positive control

orchitis in two rams and the other *M. bovigenitalium* was isolated from a buck with no apparent clinical signs.

DISCUSSION

This study confirmed of the presence of *M. bovigenitalium* and *M. arginini* in the prepuce of bucks and rams by bacteriological isolation and molecular identification. *M. bovigenitalium*, formerly known as *Mycoplasma* ovine/caprine serogroup 11, has previously been associated with abnormal sperm morphology and motility resulting in infertility in rams [1]. As its name suggests *M. bovigenitalium* has been described in cattle and it has been isolated from the reproductive tract, pneumonic, arthritic and mastitic cattle as well as aborted foetuses [9]. Lysnyansky et al.[10] identified *M. bovigenitalium* and *Mycoplasma canadense* from outbreaks of granulopapular vulvovaginitis in dairy cattle in Israel and Catania et al.[6] reported *M. bovigenitalium* from infertile cattle. There are also reports describing the isolation of *M. bovigenitalium* and *M. arginini* from the genital tract of goats [8] and semen of naturally infected asymptomatic rams [11].

Mycoplasma arginini has been isolated from a wide range of domestic animals but more commonly from sheep and goats. Rosendal [12] described it as occurring naturally in the genital tract of small ruminants. In recent years, *M. arginini* has been associated with various clinical signs in sheep and goats including pneumonia [13]. It is often thought to be a commensal or opportunistic organism and it is unusual for a mycoplasma species, in that it has been reported as occurring in many hosts, often with severe outcomes. A fatal infection due to *M. arginini* has been reported in an abattoir worker [14]. In a survey of the primary infectious agents associated with ovine ulcerative balanoposthitis and vulvovaginitis in South Africa. Kidanemariem et al.[15] identified *M. bovigenitalium* and *M. arginini* more frequently in diseased animals than healthy animals. However, Kalshingi et al.[16] isolated 34 strains of *Mycoplasma* species including *Mycoplasma bovigenitalium* and *M.arginini* from the genital tract of clinically healthy Dorper sheep and sheep with ulcerative vulvitis and balanitis.

In this study, following the bacteriological culture of *Mycoplasma* species, the PCR-DGGE method identified two *M. bovigenitalium* and one *M. arginini* from the 27 samples. Although mycoplasma culture is the standard method used by many laboratories, its success is very dependent on the laboratory receiving freshly taken samples, that have been taken aseptically, otherwise the mycoplasmas may die during transport or become overgrown by other less fastidious bacteria. This study was limited to detecting viable *Mycoplasma* species through the initial culture method. Ideally, the clinical samples should be tested immediately by PCR-DGGE, and following culture, or culture enrichment stages. The use of PCR directly on the clinical sample or from washing of the clinical sample would allow for detection of both viable and non-viable *Mycoplasma* species. Use of culture enrichment also has

the potential to further enhance the sensitivity of the PCR-DGGE method [13].

In conclusion, this study has carried out diagnostic tests for mycoplasma species that could cause infections affecting the reproductive system in male sheep and goats. It is the first report that describes the detection of *Mycoplasma* species in preputial swabs from naturally infected bucks and rams in Turkey. These results show that *Mycoplasma* species are present in the testicles of rams and bucks and were the most likely causative organisms of orchitis in the rams, which can lead to reduced fertility. In addition there is a risk that *M. bovigenitalium* can be transferred to ewes or female goats via venereal transmission. Therefore monitoring for *Mycoplasma* species by bacteriological and molecular tests such as PCR-DGGE tests are recommended for studying reproductive disorders in small ruminants. These tests can also be useful in monitoring programmes for preventing the introduction of mycoplasmas onto farms. This study highlights the usefulness of PCR-DGGE for the routine examination of ruminant samples for genital mycoplasma detection.

Bioethics and Biossecurity Committee Approval

The Scientific Ethical Committee (Uludag University. Bursa, Turkey, No: 03/07)

Declaration of Conflicting Interests

We have no conflict of interest to declare.

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REFERENCES

1. Gregory L, Rizzo H, Gaeta NC, Tortorelli G, Cardroso MV, Mettifogo E, Buzinhani M, Timenetsk J: Interference of *Mycoplasma* spp. or *Ureaplasma* spp. in ovine semen quality. *J Microbiol Res,* 2 (5): 118-122, 2012. DOI: 10.5923/j.microbiology.20120205.01

2. Razin S, Yogev D, Naot Y: Molecular biology and pathogenicity of mycoplasmas. *Microbiol Mol Biol Rev*, 62 (4): 1094-1156, 1998.

3. Office International des Epizootes (OIE): Contagious agalactia. **In,** Terrestrial Manual. 1430-1440, 2018. http://www.oie.int/en/animal-health-in-the-world/the-world-animal-health-information-system/the-oie-data-system/; *Accessed*: 25.06.2019

4. Nicholas RAJ, Khan LA, Houshaymi B, Miles RJ, Ayling RD, Hotzel H, Sachse K: Close genetic and phenotypic relatedness between *Mycoplasma* ovine/caprine serogroup 11 and *Mycoplasma bovigenitalium*. *Syst Appl Microbiol*, 25 (3): 396-402, 2002. DOI: 10.1078/0723-2020-00121

5. Chima JC, Ojo MO, Molokwu JU, Okewole PA: Characterisation of mycoplasmas isolated from genital tract infections of sheep in Nigeria. *Rev Sci Tech*, 14 (3): 865-871, 1995.

6. Catania S, Tavella A, Gobbo F, Nicholas RAJ: Isolation of *Mycoplasma bovigenitalium* from infertile dairy cattle. *Vet Rec Case Rep,* 2:e000055, 2014. DOI: 10.1136/vetreccr-2014-000055

7. Poveda J: Biochemical characteristics in mycoplasma ıdentification. **In,** Miles R, Nicholas R (Eds): Mycoplasma Protocols. 37-50, 69-78, Humana Press Inc, New Jersey, Totowa, 1998.

8. McAuliffe L, Ellis RJ, Lawes JR, Ayling RD, Nicholas RAJ: 16S rDNA PCR and denaturing gradient gel electrophoresis; a single generic test for detecting and differentiating *Mycoplasma* species. *J Med Microbiol*, 54 (8): 731-739, 2005. DOI: 10.1099/jmm.0.46058-0

9. Ruhnke H: Mycoplasmas associated with bovine genital infections. **In,** Whitford H, Rosenbusch R, Lauerman L (Eds): Mycoplasmosis in Animals. 55-61, Iowa State University Press, Ames, USA, 1994.

10. Lysnyansky I, Brenner J, Alpert N, Benjamin A, Bernstein M, Elad D, Blum S, Friedgut O, Rotenberg D: Identification of *Mycoplasma bovigenitalium* and *Mycoplasma canadense* from outbreaks of granulo-papular vulvovaginitis in dairy cattle in Israel. *Vet Rec,* 165 (11): 319-322, 2009. DOI: 10.1136/vr.165.11.319

11. Prats-Van Der Ham M, Tatay-Dualde J, de La Fe C, Paterna A, Sánchez A, Corrales JC, Contreras A, Gómez-Martín Á: Presence of *Mycoplasma agalactiae* in semen of naturally infected asymptomatic rams. *Theriogenology*, 86 (3): 791-794, 2016. DOI: 10.1016/j.theriogenology.2016.02.033

12. Rosendal S: Ovine and caprine mycoplasmas. **In,** Whitford H, Rosenbusch R, Lauerman L (Eds): Mycoplasmas in Animals: Laboratory Diagnosis. 84-92, Iowa State University Press, Ames, USA, 1994.

13. Göçmen H, Ülgen M, Çarlı **KT, Önat K, Kahya S, Özdemir Ü, Mat B:** Investigation of contagious agalactia by bacteriological and PCR methods in sheep and goats. *Kafkas Univ Vet Fak Derg* 21 (1): 75-80, 2015. DOI: 10.9775/kvfd.2014.11790

14. Yechouron A, Lefebvre J, Robson HG, Rose DL, Tully JG: Fatal septicemia due to *Mycoplasma arginini*: A new human zoonosis. *Clin Infect Dis,* 15 (3): 434-438, 1992.

15. Kidanemariam A, Gouws J, van Vuuren M, Gummow B: Ulcerative balanitis and vulvitis of Dorper sheep in South Africa: A study on its aetiology and clinical features. *J S Afr Vet Assoc,* 76 (4): 197-203, 2005. DOI: 10.4102/jsava.v76i4.426

16. Kalshingi HA, Bosman AM,Gouws J, van Vuuren M: Molecular characterisation of *Mycoplasma* species isolated from the genital tract of Dorper sheep in South Africa. *J S Afr Vet Assoc,* 86 (1): 1-11, 2015. DOI: 10.4102/jsava.v86i1.1199