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Case Report / Olgu Sunumu

Diagnosis of Mycoplasma agalactiae from Various Specimens of Goats

Keçilerin Çeşitli Örneklerinden Mycoplasma agalactia'nın Teşhisi

Dilek ÖZTÜRK[1]*, Sibel YAMAN[1]

[1]Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Microbiology, Burdur, Türkiye

Abstract: The aim of this study was to determine the presence of Mycoplasma infection by bacteriological methods and to reveal the prevalence of the disease in a goat farm with pneumonia, mastitis and arthritis symptoms and deaths. This study was carried out in a Saanen goat farm in Isparta province between January 2015 and January 2017. Samples (milk, intra-articular fluid, internal organs of deceased animals) were brought to XXXX University, Faculty of Veterinary Medicine Microbiology Laboratory and examined by bacteriological methods. Blood samples were collected from 813 goats in aged 1-6 years in the goat farm. Blood sera were tested for *Mycoplasma agalactiae* with a commercial ELISA kit (*Mycoplasma agalactiae* Antibody test kit, IDEXX, France) according to the kit instructions. *Mycoplasma* colonies were isolated from all samples. As a result of serological examination, 83 (10.2%) animals were positive, 9 (1.1%) animals were suspicious, and the rest were negative by ELISA. The seropositivity of *M. agalactiae* was ranged from 5.1 % to 28.7% according to age, and it was found quite high in three-year-old animals. With this study, it was concluded that the seroprevalence of M. agalactiae in goats is quite high in goats aged three years and older, ELISA positive animals can be detected in asymptomatic animals, and ELISA test can be used to determine the seroprevalence of the disease in herds.

Keywords: ELİSA, Goat, *Mycoplasma agalactiae.*

Öz: Bu çalışmanın amacı, pnömoni, mastitis ve artritis sempomları ile birlikte ölümler görülen bir keçi çiftliğinde Mycoplasma enfeksiyonunun varlığını bakteriyolojik yöntemlerle belirlemek ve hastalığın prevalansını ortaya koymaktır. Bu çalışma 2015 Ocak- 2017 Ocak ayları arasında Isparta ilinde bir Saanen keçi çiftliğinde yapıldı. XXXX Veteriner Fakültesi Mikrobiyoloji Laboratuvarı'na getirilen örnekler (hangi örnekler) bakteriyolojik metotlarla incelendi. Ayrıca keçi çiftliğinde yaşları 1-6 arasında değişen 813 keçiden kan örnekleri toplandı. Kan serumları *M. agalactiae* varlığı yönünden ticari bir ELISA kiti (*Mycoplasma agalactiae* Antikor test kiti, Idexx, France) ile kit prospektüsüne uygun olarak test edildi. Örneklerden Pleura Pneumonia Like Organism (PPLO) besiyerine ekimler yapıldı ve *Mycoplasma* spp. kolonileri tüm örneklerden saf olarak izole edildi. Serolojik muayene sonucunda ELISA ile 83 (%10.2) hayvan pozitif, 9 (%1.1) hayvan şüpheli ve geri kalanı negatif olarak saptandı. *M. agalactiae* enfeksiyonunun seropozitifliği, yaşlara göre değerlendirildiğinde, %5.1 ile %28.7 arasında değiştiği, üç yaşındaki hayvanlarda oldukça yüksek olduğu belirlendi. Bu çalışma ile keçilerde *M. agalactiae*'nın seroprevalansının üç yaş ve üzerindeki keçilerde oldukça yüksek olduğu, semptom göstermeyen hayvanlarda ELISA pozitif hayvanların tespit edilebileceği, sürülerden hastalığın seroprevalansını belirlemede ELISA testinin kullanılabileceği kanaatine varıldı.

Anahtar Kelimeler: ELİSA, Keçi, *Mycoplasma agalactiae.*

*Corresponding author : Dilek ÖZTÜRK
e-mail : dozturk@mehmetakif.edu.tr
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Introduction

Mycoplasma agalactiae (*M. agalactiae*), belonging to the genus *Mycoplasma* in the class Mollicutes, is a Gram-negative, non-spored, non-capsulated, usually immobile, polymorphic bacterium with a very small genome and no cell wall that causes infectious agalactiae disease in sheep and goats (Kumar et al., 2014; Reji et al., 2018). Instead of a cell wall, they have a three-layered unit membrane composed of lipids, carbohydrates and proteins (Jay and Tardy, 2019; Kumar et al., 2014).

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Contagious agalactia is an acute, subacute or chronic disease characterized by mastitis, kerataconjunctivitis and arthritis. The causative agent is isolated from milk, joint fluid, eye and nasal discharges, feces, urine samples of infected animals and internal organs of dead animals. The disease is transmitted to susceptible animals by direct and indirect routes, usually through the digestive tract and less commonly through the respiratory and ocular mucosa (Göçmen et al., 2015; Kumar et al., 2014; Reji et al., 2018). Infectious agalactia has been known for more than 200 years. The presence of this disease was first reported in Italy in 1816 (Jay and Tardy, 2019). Infectious agalactia, which is included in the list of notifiable diseases of the World Organization for Animal Health (OIE), is seen in many parts of the world, especially in Mediterranean countries (Assunçao et al., 2007; Göçmen et al., 2015; Kumar et al., 2014; OIE, 2008). Infectious agalactia is known to vary from asymptomatic to chronic forms (Göçmen et al., 2015; Keskin, 2018). In acute form, widespread fever and in some cases, neurological symptoms are observed, while in chronic form, anorexia, weakness, lagging behind the flock, loss of appetite, and decreased milk production are observed (Reji et al., 2018). The incubation period of the disease, which can vary between 1 and 8 weeks, has a morbidity rate of 30-60% and a mortality rate of 20%. However, during the suckling period, the mortality rate can increase up to 40-70% due to the occurrence of septicemia in animals. Sheep in the nursing period are more susceptible to the disease (Bohach et al., 2021).

The aim of this study was to determine the presence of Mycoplasma infection by bacteriological methods and to reveal the prevalence of the disease in a goat farm with pneumonia, mastitis and arthritis symptoms and deaths.

Material and Method

This study was conducted in a Saanen goat farm located in Isparta province between 2015 and 2017. The goats on the farm had not been vaccinated for mycoplasma. Samples were collected from milk, joint contents of sick goats and internal organs of deceased animals. These samples were transported under cold chain conditions to the Department of Microbiology, Faculty of Veterinary Medicine, XXXX University. Additionally, blood samples were obtained from 813 goats with ages ranging from 1 to 6 years, and they were transported to the laboratory under cold chain conditions for serological examinations.

Bacteriological Culture

The milk, joint contents, and internal organ samples brought to the laboratory were inoculated onto Blood agar (Oxoid, UK) with 7% defibrinated sheep blood, MacConkey agar (Merck, Germany), and PPLO agar supplemented with *Mycoplasma* supplement (Oxoid, UK). Petri dishes were incubated at 37°C with 5% CO2 in an incubator for 5-7 days. To separate the isolated *Mycoplasma spp.* colonies from other bacterial colonies, subcultures were performed on PPLO agar without *Mycoplasma* supplement.

ELISA

The serum samples were obtained from the 813 blood samples brought to the laboratory by centrifuging at 3000 rpm for 5 minutes. The serum samples were tested using an indirect ELISA (*Mycoplasma agalactiae* Antibody test kit, Idexx, France) according to the kit procedure. The OD (optical density) values of the samples were determined using an ELISA reader with a 450 nm filter.

Results

Mycoplasma spp. colonies were isolated in pure form from the milk, joint contents, and internal organ samples sent to the laboratory (Figure 1). When these colonies were subcultured on PPLO agar without supplement, PPLO colonies were observed. These colonies were considered as *Mycoplasma spp.*

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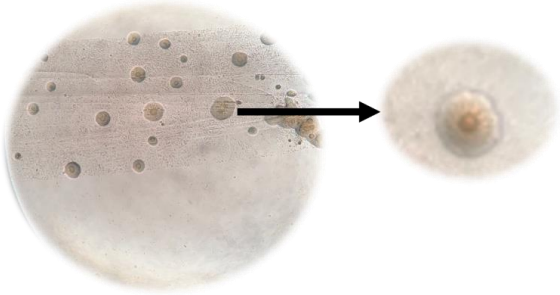

Figure 1. Microscopic image of *Mycoplasma spp.* colonies.

In this study, 83 (10.2%) animals were found to be positive, 9 (1.1%) animals were considered suspicious, and the rest of the animals were negative by ELISA (Table 1). When evaluating the *M. agalactiae* seropositivity according to age, it was found to range from 5.1% to 28.7%, with a significant increase in three-year-old animals. The seropositive animals identified animals were sent to slaughter, while the suspicious goats were placed in a separate area and tested again with ELISA after one month. The positive animals were sent for slaughter. The entire herd was screened with ELISA every 6 months until all animals tested negative, and the positively identified animals were removed from the herd.

Table 1. Distribution of *M. agalactiae* infection by age.

	Age of animals					
ELISA	**1 year**	**2 year**	**3 year**	**4 year**	**5+ year**	**Total**
Positive	12 (%5.1)	5 (%2.3)	42 (%28.7)	13 (%12.2)	11 (%10.09)	83 (%10.2)
Negative	220 (%93.6)	208 (%95.8)	104 (%71.2)	92 (%86.7)	97 (%88.9)	721 (%88.6)
Suspect	3 (%1.2)	4 (%1.8)	0	1 (%0.94)	1 (%0.91)	9 (%1.1)
Total	235	217	146	106	109	813

Discussion

Contagious agalactia, characterized by deaths in young animals, abortions in pregnant animals, and most importantly, decreased milk production, causes significant economic losses in goat farming. Rapid and accurate diagnosis of *M. agalactiae* is crucial for the control and prevention of this disease (Keskin, 2018). Various samples such as milk, blood, serum, nasal swabs, joint fluid, and vaginal swabs are used for the diagnosis of *M. agalactiae*. Studies on *Mycoplasma* have been reported in many countries worldwide (Azevedo et al., 2006; Bandeira et al., 2008; Lin et al., 2022; Mohan and Uzoukwu, 1985). In the study conducted by Kinde et al. (1994) in California, the introduction of new goats without showing any clinical symptoms into a farm with 600 goats resulted in the occurrence of arthritis, polyarthritis, and mastitis in the animals within a short period of 4 weeks, followed by sudden deaths. Milk samples and postmortem examinations of the dead animals were analyzed using bacteriological methods, and a diagnosis of *M. agalactiae* was made.

Azevedo et al (2006) investigated the causes of death of animals in a goat farm in Brazil between 2001 and 2002 in which mastitis and polyarthritis in goats, polyarthritis and conjunctivitis symptoms in kids and lambs were observed, first by bacteriological methods and then by molecular methods. 89 *Mycoplasma* spp. isolates were obtained

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from 107 samples (11 milk, 8 joint fluid, 22 nasal fluid, 66 ear fluid).

In a study conducted in and around Ankara, the first isolation of *M. agalactiae* from milk, joint fluid, nasal discharge and conjuctival fluid samples was realized (Beşe and Arda, 1968). Kızıl and Ozdemir (2006) in Elazığ, they collected milk samples from 47 goats showing symptoms of fever, mastitis, and arthritis, as well as from 20 asymptomatic goats. They investigated the samples using both bacteriological and molecular methods and reported that 17 of the samples were diagnosed with *M. agalactiae* using both methods. Ongor et al. (2011) in eastern Turkey, they collected 692 nasal swab samples from 44 goat herds with nasal discharge. They reported isolation of *Mycoplasma* sp. in 6 of the samples. In another study conducted in same region. Çetinkaya et al. (2009) reported that *Mycoplasma capricolum subsp. capripneumoniae* was isolated and identified from 12 of 32 lung samples collected from 10 different sheep and goat flocks. The samples were subjected to bacteriologic and molecular investigations. In studies conducted in Turkey, reports have been made of *M. agalactiae* in cases of mastitis, arthritis, keratitis, pneumonia, nasal discharge, and anorexia symptoms in cattle (Şababoğlu et al., 2018), sheep (Beşe and Arda, 1968; Göçmen et al., 2015), and goats (Çetinkaya et al., 2009; Kızıl and Ozdemir, 2006; Ongon et al., 2011). This study, similar to the reported studies, isolated *Mycoplasma spp*. colonies on PPLO agar from goats showing mastitis and arthritis symptoms, as well as from the internal organs of deceased animals. This indicates that *Mycoplasma* spp. can be easily isolated from samples taken from goats exhibiting clinical symptoms of contagious agalactia. The isolation of the pathogen from all animals suggests that *Mycoplasma* spp. may be responsible for the deaths in the goat farm. In this study, the serological diagnosis of the infection was performed using ELISA. It was found that out of the animals tested, 83 (10.2%) were positive, 9 (1.1%) were suspected, and the remaining animals were negative for the infection. It was determined that the seroprevalence of *M. agalactiae* is quite high in goats aged 3 years and above, ranging from 5.1% to 28.7% depending on the age. According to this finding, it is believed that older goats in the farm are more susceptible to the disease. In the reported studies (Verbisck-Bucker et al., 2008; Bohach et al., 2021), contagious agalactia was reported to be more common in younger goats. The reason for this is attributed to the fact that female and young animals are usually kept in groups (Verbisck-Bucker et al., 2008). Verbisck-Bucker et al. (2008) conducted a study to investigate the epidemiology of *M. agalactiae* and found that season, gender, secondary agents, age, and reproductive periods were influential factors in the susceptibility of animals to the disease. They concluded that female goats and young animals were more susceptible to the disease. According to Bohach et al. (2021), who conducted a study in Ukraine between 2016 and 2018, they examined blood samples collected from 1,964 sheep and 1,484 goats, including different age groups, using ELISA, and reported that the most susceptible age group to *Mycoplasma spp*. infection was 1-year-old animals. In this study, the higher seroprevalence of *M. agalactiae* in 3-year-old animals compared to other age groups was thought to be associated with the fact that all animals in the herd were kept in a common area on the farm.

M. agalactiae is commonly diagnosed using culture techniques, but the time required to obtain results is considered a significant disadvantage. With advancements in technology, PCR methods have been employed. Assunçao et al. (2007) conducted a study evaluating the applicability of PCR for *M. agalactiae* diagnosis and demonstrated that it is faster and more sensitive compared to culture methods. Therefore, PCR has become the preferred method for *M. agalactiae* diagnosis today (Reji et al. 2018; Göçmen et al. 2015). In Turkey, there are few studies on *M. agalactiae*, and they are generally not focused on determining herd prevalence. The use of ELISA is important in monitoring herd health and conducting epidemiological studies, as it provides both diagnostic capabilities and time-saving advantages. The aim of this study was to establish the seroprevalence of Mycoplasma agalactia,

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therefore molecular diagnostic methods were not used in this study.

In conclusion, this study has determined that *Mycoplasma* spp. can cause symptoms such as pneumonia, mastitis, and arthritis in goats, leading to fatalities. It has been confirmed that the pathogens can be isolated from internal organs, milk, and joint fluid using the culture method. It was concluded that seroprevalence is high in goats aged three years and older, and that ELISA can detect ELISA-positive animals even in asymptomatic individuals. It was also suggested that ELISA testing can be used in the eradication of the disease from herds. The high seroprevalence of *M. agalactiae* indicates that the disease is endemic in the herd. The eradication of the disease from the herd is possible through the identification and removal of infected animals from the flock. There is a need for more studies to be conducted in Turkey and for the identification of strains causing Contagious Agalactia, in order to establish targeted prevention and control programs.

Ethics Approval

I hereby declare that Ethics Committee Approval is not required for the publication given below prepared by the study team.

Conflict of Interest

The authors declare that they have no conflict of interest.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal interests.

Authorship Contributions

During the study's preparation, all authors contributed equally.

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