RESEARCH ARTICLE Indian Journal of Animal Research, Volume 56 Issue 3: 358-361 (March 2022)

An Investigation of Contagious Agalactia Disease of Sheep and Goats in Isparta and Afyonkarahisar in Turkey

Ülkü Karatekeli[1], Beytullah Kenar[2]

10.18805/IJAR.BF-1428

ABSTRACT

Background: Contagious agalactia causes significant economic losses. The aim of this study is to investigate the presence of contagious agalactia disease in cities of Isparta and Afyonkarahisar, Turkey.

Methods: The study includes 45.500 animals in 220 ovine enterprises and samples were taken from those suspected of contagious agalactia disease. 202 animals in the 21 ovine enterprises comprised of 139 goats, 56 sheep, 3 kid goats, 2 goats, 2 lambs in total suspected of the disease were sampled. A total of 289 samples were collected, including 91 milk samples, 28 nasal swabs, 101 eye swabs, 8 joint fluids and 61 ear swabs. The isolates obtained after incubation were identified with polymerase chain reaction by using specific primers to assess film and spot formation, glucose fermentation, growth inhibition tests.

Result: Three *Mycoplasma* spp. isolates obtained from 28 nasal swabs turned out to be negative for *M. agalactiae* after PCR analysis. Colony morphology, biochemical tests and growth inhibition tests revealed that one agent was *M. arginine* and the two factors were identified as *M. ovipneumoniae* with centerless colony morphology. The obtained results were confirmed with the polymerase chain reaction. None of the four factors causing contagious agalactia were isolated and identified.

Key words: Identification, Isolation, *Mycoplasma,* PCR, Small ruminants.

INTRODUCTION

Mycoplasma have a very wide variety of hosts, namely small ruminants (Marenda *et al.* 2005; Manimaran and Singh, 2018), cattle (Goswami *et al.* 2019; Waseem *et al.* 2020) Poultry (Manimaran and Singh, 2018; Rajkumar *et al.* 2019). Contagious agalactia occurs in many parts of the world, especially in the Mediterranean and in Turkey (Amores *et al.* 2011; Birben, 2020; Ozturkler and Otlu, 2020) Basin.

It is on the list of mandatorily notifiable diseases of the International Office of Epizooties (OIE). Contagious agalactia causes high morbidity in sheep and goat breeding and serious economic losses with the treatment costs of the disease (OIE Terrestrial Manuel, 2018; Tardy *et al.* 2012).

Mycoplasma agalactiae (Ma), *Mycoplasma capricolum* subsp. *Capricolum* (Mcc), *Mycoplasma mycoides* subsp. *capri* (Mmc) and *Mycoplasma putrefaciens* (Mp) which are strains of contagious agalactia have been described several times in cattle as symptomatic or asymptomatic (Amores *et al.* 2011; Gomez-Martin *et al.* 2013; Heller *et al.* 2015).

The most prominent etiological agent of contagious agalactia disease is *M. agalactiae*. Clinical manifestations of contagious agalactia are arthritis, mastitis, keratoconjunctivitis, sporadic or epidemic abortions, emaciation, respiratory system symptoms ranging from cough to dyspnea (Gomez-Martin *et al.* 2013). With the birth of lambs in the spring, the sudden spread of the disease in lactating ewes has been shown and newborns have been reported to be infected with infected colostrum or milk (OIE Terrestrial Manuel, 2018). Epidemiologically, early diagnosis of the disease is important (Pooladgar *et al.* 2015; Razin *et al.* 1998). Using immunomagnetic PCR methods to detect *M. agalactiae* in milk samples gives faster results than using the culture enrichment procedure (Cillara *et al.* 2015).

A multiplex PCR assay capable of simultaneously detecting *M. agalactiae*, Mcc and Mmc has been described. In addition, a PCR based on the lpdA gene and a restriction fragment length polymorphism PCR (RFLP-PCR) method has been described to distinguish between Mmc and Mcc (Gil *et al.* 2003).

The aim of this study was to investigate the presence of the disease for the first time by using both cultural and molecular methods on the field strains that cause contagious agalatiae in Isparta and Afyonkarahisar provinces in Turkey.

MATERIALS AND METHODS

Animals and samples

For this investigation, 45,500 goats and sheep were scanned from 220 ovine farms in the Isparta and Afyonkarahisar

[1]District Directorate of Agriculture and Forestry Eğirdir - Isparta, Turkey.

[2]Department of Microbiology, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey.

Corresponding Author: Beytullah Kenar, Department of Microbiology, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey. Email: bkenar@aku.edu.tr

How to cite this article: Karatekeli, Ü. and Kenar, B. (2022). An Investigation of Contagious Agalactia Disease of Sheep and Goats in Isparta and Afyonkarahisar in Turkey. Indian Journal of Animal Research. 56(3): 358-361. DOI: 10.18805/IJAR.BF-1428.

Submitted: 18-08-2021 **Accepted:** 14-10-2021 **Online:** 09-12-2021

regions. Clinical and bacteriological examinations were performed on a total of 202 animals, 144 goats and 58 sheep, from 21 small ruminant farms with mastitis, keratoconjunctivitis, limping, swelling/arthritis in the joints, abortus, runny nose and pneumonia symptoms for the isolation and identification of Mycoplasma species. This study was carried out during 2018-2019.

A total of 289 samples were taken, including 91 milk samples (68 goats and 23 sheep), 101 eye swabs (47 goats and 54 sheep), 61 ear swabs (59 goats and 2 sheep), 28 nose swabs (13 goats and 15 sheep) and 8 joint fluid samples (8 sheep).

Bacterial isolation and identification of *Mycoplasma* spp.

Eye, nose and ear swabs taken from animals with suspected disease were collected in Hayflik's transport medium, joint fluid was drawn into a sterile syringe and milk samples were filled into sterile tubes to reach the laboratory in a cold chain as soon as possible. 10^{-1} dilutions of the suspicious samples were prepared in a liquid medium and/or cultivated directly into the Mycoplasma agar. Petri dishes were incubated at 37°C in a 5-10% CO_2 setup. Solid media were examined for growth with a stereo microscope (X5-50 magnification) every 2-3 days. Negative petri dishes were destroyed after 14 days of incubation. The morphology of observed colonies was examined, the part containing the colony was cut as a block and transferred into a liquid medium. Isolates were identified by using biochemical tests (film and spot formation, glucose fermentation test) and the growth inhibition test (Poveda, 1998).

PCR

This test was carried out at the Ministry of Agriculture and Forestry of the Republic of Turkey, Istanbul Pendik Veterinary Control Institute Mycoplasma Reference Diagnostic Laboratory. *M. agalactiae* PCR was performed for 3 isolates obtained using the primer sequences indicated in Table 1.

RESULTS AND DISCUSSION

At the end of the incubation period, a total of 289 samples were examined under a stereo microscope for typical 'fried egg' colony formation. *Mycoplasma* spp. was determined in only 3 (1.03) out of 289 samples. *Mycoplasma* spp. could not be isolated from any samples other than 3 goat nasal swabs (Table 2).

The results of the biochemical tests revealed that film and spot formation was negative and the glucose fermentation test indicated that two colonies gave positive results and one colony gave negative results. Colony morphologies were examined and as a result of biochemical tests and growth inhibition tests, *Mycoplasma* spp. 2 of the 3 (1.03%) isolates evaluated as *M. ovipneumoniae* (0.67%) and 1 isolate were identified as *M. arginine* (0.36%). In addition, *M. agalactiae* PCR was applied to 3 isolated samples and the isolates were found negative for agalactiae (Table 3).

Valsala *et al.* (2017) examined a total of 244 goat lung tissue samples with pneumonia collected for 10 years (2002-2013) and according to the results of biochemical tests, *M. arginine* (13/244, 5.3%) was the most common isolate. In the phylogeny analysis performed in the same study, they reported that the sequences of all these *M. arginine* isolates were >98% identical compared to the standard *M. arginine* (ATCC 23243) strains and that *M. agalactiae* was isolated in only one sample.

In a thesis study, Göçmen (2014) investigated the existence of *Mycoplasma* spp. in sheep and goats in the Marmara Region of Turkey for which a total of 339 samples, including 162 milk samples, 147 eye swabs, 15 joint fluid samples, 11 nasal swabs and 4 lung tissue samples were examined by bacteriological and molecular methods. As a result of the biochemical tests and growth inhibition tests applied in the bacteriological examination, *Mycoplasma* spp. was identified in 25 of 29 (8.5%) isolates evaluated as *M. agalactiae* (86.20%). She reported that only 1 isolate each was identified from the *M. ovipneumoniae* and *M. arginine* (6.89%) cases according to the biochemical test results.

Ozturkler and Otlu (2020) conducted a study in which *Mycoplasma* spp. were investigated by cultural and molecular methods in the lung tissue of 250 sheep with pneumonia and 30 healthy sheep purchased from slaughterhouses and butchers in Kars province of Turkey. *Mycoplasma* was isolated in only 26 (10.4%) of 250 sheep lung tissues with pneumonia. They reported that *M. ovipneumoniae* isolated from *Mycoplasma* spp.'s was encountered the most with 12 (46.15%) samples, followed by *M. arginine* with 4 (15.38%) samples.

In Egypt, Halium *et al.* (2019) carried out a study for *Mycoplasma* spp. in sheep and goats. They examined a total of 335 samples, including 142 nasal swabs, 167 lung tissues with pneumonia, 18 tracheal bifurcations and 8 bronchial fluids. They reported that a total of 24 *Mycoplasma* were isolated and they confirmed by PCR that 10 of these 24 isolates were *M. arginine* and 4 were *M. ovipneumoniae*.

Zhao *et al.* (2021) isolated *M. ovipneumoniae* in 4 out of 6 lung tissues in a study they conducted on 6 sheep lungs and a total of 824 nasal swab samples from animals that had died from pneumonia in 4 different regions in China between 2018 and 2020. They confirmed by PCR that 336 (40.78%) of 824 nasal swab specimens tested in the same study were *M. ovipneumoniae*. They also reported that they detected *M. arginine* in this study and that *M. arginine* is

Table 1: *Mycoplasma agalactiae* specific primer sequences.

Primer	Sequence	PCR product size
MAPol-1F:	5′-CATTGAACCTCTTATGTCATTTACTTTG-3′	265 bp
MAPol-5R:	5′-CTATGTCATCAGCTTTTGGGTGA-3′	

Table 2: *Mycoplasma* spp. positivity rate in terms of animal species, sample type and numbers.

Animal species	Type of sample	Number of samples	Number of *Mycoplasma* spp. (positive samples) (%)
Goat (n=144)	Milk	68	0
	Eye swabs	47	0
	Ear swabs	59	0
	Nose swabs	13	3
Sheep (n=58)	Milk	23	0
	Eye swabs	54	0
	Ear swabs	2	0
	Nose swabs	15	0
	Joint fluid	8	0
Total (n=202)		289	3 %1.03

Table 3: Results of isolated *Mycoplasma* spp. identification studies.

Mycoplasma spp.	*M. agalactiae* PCR	Reproduction inhibition test	Film and spot formation	Glucose fermentation	Colony morphology
M. arginine	-	+	-	-	Centered
M. ovipneumoniae	-	+	-	+	Centerless

usually found together with *M. ovipneumoniae*, but it is not pathogenic. Similarly, in our study, *M. arginine* was identified in 1 sample while *M. ovipneumoniae* was identified in 2 samples.

CONCLUSION

Literature reviews indicate that the disease still continues to be seen in our country. The isolation of *M. ovipneumoniae* and *M. arginine* from animals with clinically suspected contagious agalaxia made us think that the effects of these factors on the disease should be investigated. Controlling animal movements, implementing proper milking hygiene, prioritizing the hygiene of milking tools and equipment as well as the hygiene of the operation, checking the herd with serological methods at regular intervals, removing sick or suspected animals from the herd, implementing planned vaccination programs and parasite control can be counted among measures that can be taken against contagious agalaxia. In addition to *M. agalactiae* causing the disease, the incidence of *M. ovipneumoniae* and *M. arginine* agents in our country should be investigated and these factors should be taken into consideration in vaccine development studies. It has been concluded that it would be beneficial to include the disease in the list of notifiable diseases to determine the true prevalence of the disease.

ACKNOWLEDGEMENT

This study was summarized in the Masters' Thesis titled "Investigation of the Presence of Contagious Agalaxia Disease in Sheep and Goats in the Region of Isparta and Afyonkarahisar" and was supported by the Afyon Kocatepe University Scientific Research Projects Coordination Unit (Project no: 2019-053).

This study was produced from "Investigation of the Presence of Contagious Agalaxia Disease in Sheep and Goats in the Region of Isparta and Afyonkarahisar" master's thesis and was presented in XIV. National Veterinary Microbiology Congress (With International Participation) Konya, Turkey, 13-16 October 2020.

Molecular studies of this research were carried out in the Ministry of Agriculture and Forestry, Pendik Institute of Veterinary Control, Mycoplasma Reference Laboratory.

Ethical approval

The necessary permission for milk, joint fluid, eye, ear and nose swab samples taken from animals during this study was approved by Afyon Kocatepe University Animal Experiments Local Ethics Committee (HADYEK) with the decision dated 14/02/2018 and numbered 49533702/01.

REFERENCES

Amores, J., Gomez-Martin, A., Corrales, J.C., Sanchez, A., Contreras, A. and De La Fe, C. (2011). Presence of contagious agalactia causing mycoplasmas in Spanish goat artificial insemination centres. Theriogenology. 75: 1265-1270. doi: 10.1016/j.theriogenology.2010.11.040.

Birben, N. (2020). Determination of contagious agalactia in sheep and goats and investigation of antibiotic susceptibility of *Mycoplasma* spp. positive isolates. Indian Journal of Animal Research. DOI: 10.18805/ijar.B-1253.

Cillara, G., Manca, M.G., Longheu, C. and Tola, S. (2015). Discrimination between *Mycoplasma mycoides* subsp. *capri* and *Mycoplasma capricolum* subsp. *capricolum* using PCR-FLP and PCR. The Veterinary Journal. 205: 421-423. DOI: 10.1016/j.tvjl.2015.05.013.

Gil, M.C., Pena, F.J., Hermoso De Mendoza, J. and Gomez, L. (2003). Genital lesions in an outbreak of caprine contagious agalactia caused by *Mycoplasma agalactiae* and *Mycoplasma putrefaciens*. Journal of Veterinary Medicine. B, 50: 484-487. doi: 10.1046/j.0931-1793.2003.00709.x.

Gomez-Martin, A., Amores, J., Paterna, A. and De La Fe, C. (2013). *Contagious agalactia* due to *Mycoplasma* spp. in small dairy ruminants: Epidemiology and prospects for diagnosis and control. The Veterinary Journal. 198: 48-56. doi: 10.1016/j.tvjl.2013.04.015.

Goswami, P., Banga, H.S. and Mahajan, V. (2019). Pathological description of naturally occurring *Mycoplasma bovis* associated pneumonia in bovine calves. Indian Journal of Animal Research. 53: 799-806. DOI:10.18805/ijar.B-3570.

Göçmen, H. (2014). Marmara Bölgesinde Koyun ve Keçilerde *Mycoplasma agalactiae*'nın Bakteriyolojik ve Moleküler Yöntemler ile Araştırılması. Doktora Tezi, Uludağ Üniversitesi, Sağlık Bilimleri Enstitüsü, Bursa, Turkey. [Turkish].

Halium, M.M.A., Salib, F.A., Marouf, S.A. and Massieh, E.S.A. (2019). Isolation and molecular characterization of *Mycoplasma* spp. in sheep and goats in Egypt. Veterinary World. 12: 664-670. doi: 10.14202/vetworld.2019.664-670.

Heller, M., Schwarz, R., Noe, G., Jores, J., Fischer, A., Schubert, E. and Sachse, K. (2015). First human case of severe septicaemia associated with *Mycoplasma capricolum* subsp. *capricolum* infection. Journal of Medical Microbiology Case Reports. 2: 1-3. DOI 10.1099/jmmcr.0.000101.

Manimaran, K. and Singh, V.P. (2018). Rapid detection of infection due to *Mycoplasma mycoides* subsp *capri* in experimental goats by PCR by assay. Indian Journal of Animal Research. 52: 758-760. DOI: 10.18805/ijar.B-3010.

Marenda, M.S., Sagne, E. Poumarat, F and Citti, C. (2005). Suppression subtractive hybridization as a basis to assess *Mycoplasma agalactiae* and *Mycoplasma bovis* genomic diversity and species-specific sequences. Microbiology. 151: 475-489. doi: 10.1099/mic.0.27590-0.

OIE Terrestrial Manuel (2018). Chapter 3.7.3 Contagious agalactia, 1430-1440. Date of acces: 10 August 2021, https://www.oie.int/fileadmin/Home/fr/Health_standards/tahm/3.07.03_CONT_AGALACT.pdf.

Ozturkler, O. and Otlu, S. (2020). A phylogenetic analysis of *Mycoplasma* strains circulating in sheep pneumonia in the Kars region of Turkey. Turkish Journal of Veterinary and Animal Sciences. 44: 805-813. doi:10.3906/vet-2001-59.

Pooladgar, A.R., Looni, R., Ghaemmaghami, S.H., Pourbakhsh, A., Ashtari, A. and Ali Shirudi, A. (2015). Isolation and identification of *Mycoplasma agalactiae* by culture and polymerase chain reaction (PCR) from sheep of Khuzestan province, Iran. Archives of Razi Institute. 70: 21-27. DOI: 10.7508/ari.2015.01.004.

Poveda, J.B. (1998). Biochemical Charactristics in *Mycoplasma* Identification. In: Methods in Molecular Biology. [Miles R., Nicholas RA.] Editors. Mycoplasma Protocols, 1st ed. Humana Press Inc., Totowa, NJ. New Jersey, USA.

Rajkumar, S., Reddy, M.R. and Somvanshi, R. (2019). Molecular typing of Indian *Mycoplasma gallisepticum* isolates. Indian Journal of Animal Research. 53: 1645-1650. DOI: 10.18805/ijar.B-3715.

Razin, S., Yogev, D. and Naot, Y. (1998). Molecular biology and pathogenicity of mycoplasmas. Microbiology Molecular Biology Reviews. 62: 1094-1156.

Tardy, F., Baranowski, E., Nouvel, L. X., Mick, V., Manso-Silvàn, L., Thiaucourt, F., Thébault, P., Breton, M., Sirand-Pugnet, P., Blanchard, A., Garnier, A., Gibert, P., Game, Y., Poumarat, F. and Citti, C. (2012). Emergence of atypical *Mycoplasma agalactiae* strains harboring a new prophage and associated with an alpine wild ungulate mortality episode. Applied and Environmental Microbiology. 78: 4659-4668. https://doi.org/10.1128/AEM.00332-12.

Valsala, R., Rana, R., Remesh, A.T. and Singh, V. P. (2017). *Mycoplasma arginini*: High frequency involvement in goat pneumonia. Turkish Journal of Veterinary and Animal Sciences. 41: 393-399. doi:10.3906/vet-1604-75.

Waseem, R., Muhee, A., Malik, H.U., Akhoon, Z.A., Khusheeba Munir, S.U. and Nabi, S.T. (2020). Isolation and identification of major mastitis causing bacteria from clinical cases of bovine mastitis in Kashmir Valley. Indian Journal of Animal Research. 54: 1428-1432. DOI: 10.18805/ijar.B-3848.

Zhao, J., Du, Y., Song, Y., Zhou, P., Chu, Y. and Wu, J. (2021). Investigation of the prevalence of *Mycoplasma ovipneumoniae* in Southern Xinjiang, China. Journal of Veterinary Research. 65: 155-160. doi: 10.2478/jvetres-2021-0021.