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Investigation of *Mycoplasma bovis* in Bovine Lung Samples with Pneumonia by Real Time PCR *

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The aim of this study was to investigate *Mycoplasma (M.) bovis* in bovine lungs with pneumonia by real time polymerase chain reaction (PCR). In this study, 100 lung tissue samples taken from cattle with pneumonia that were macroscopically diagnosed and brought to Adana Veterinary Control Institute were used. Real time PCR was used for the detection of *M. bovis* in the samples and conventional bacteriological culture method was used for the detection of other bacterial agents. *M. bovis* was detected in 52 of 100 bovine lung samples by real time PCR. In 58 of these samples, various bacteriological agents were isolated and identified but bacterial agent was not isolated from 42 of lung samples by conventional bacteriological methods. *Escherichia coli* was detected in the highest rate (25%) after *M. bovis* while *Pasteurella multocida, Klebsiella pneumoniae, Sphingomonas paucimobilis, Mannheimia haemolytica, Staphylococcus epidermid*is and *Staphylococcus* spp. were isolated from 13%, 7%, 7%, 4%, 1% and 1% of the samples, respectively. The findings of the study indicated that *M. bovis* was an important causative agent of respiratory diseases in cattle, and that the presence of other bacterial agents should be considered in the diagnosis and treatment of the disease.

Key Words: *Bovine, lung, Mycoplasma bovis, real time PCR*

Pnömonili Sığır Akciğer Örneklerinde *Mycoplasma bovis*'in Real Time PCR ile Araştırılması

Bu çalışmada, pnömonili sığır akciğerlerinde *Mycoplasma (M.) bovis*'in varlığı, gen spesifik primerlerin kullanıldığı real time polimeraz zincir reaksiyonu (PCR) yöntemi ile araştırıldı. Makroskobik olarak pnömoni tanısı koyulan 100 adet sığır akciğer örneğinin 52'sinde real time PCR ile *M. bovis* saptandı. Örneklerin 58'inde konvansiyonel bakteriyolojik yöntemler ile çeşitli bakteriyel etkenlerin varlığı tespit edildi. Çalışmada *M. bovis*'den sonra en yüksek oranda (%25) *Escherichia coli* tespit edilirken, *Pasteurella multocida, Klebsiella pneumoniae, Sphingomonas paucimobilis, Mannheimia haemolytica, Staphylococcus epidermidis* ve *Staphylococcus* spp. örneklerin sırasıyla %13, %7, %7, %4, %1 ve %1'inden izole edildi. Akciğer örneklerinin 42'sinden ise konvansiyonel bakteriyolojik yöntemler ile bakteriyel etken izole edilmedi. Çalışmadan elde edilen bulgular ile *M. bovis*'in sığırlarda solunum sistemi hastalıklarına neden olan önemli bir etken olduğu belirlenmekle birlikte hastalığın teşhis ve tedavisinde diğer bakteriyel etkenlerin varlığının da göz önünde bulundurulması gerektiği kanaatine varıldı.

Anahtar Kelimeler: *Sığır, akciğer, Mycoplasma bovis, real time-PCR*

Introduction

Respiratory system diseases, which are frequently encountered in cattle in Türkiye as well as all over the world, are an important health problem in terms of animal health (1-3). Various predisposing factors such as infectious agents, care and feeding are important in the formation of respiratory system diseases. While viral agents such as Bovine Respiratory Syncytial Virus, Parainfluenza Virus Type 3, Infectious Bovine Rhinotracheitis, Bovine Viral Diarhea and Bovine Herpes Virus are common in the disease, bacterial agents include *Pasteurella multocida, Mannheimia haemolytica, Histophilus somni* and *Mycoplasma* spp. draws attention. Among bacterial agents, especially mycoplasmas cause serious economic losses. *Mycoplasma bovis (M. bovis)* is the most frequently isolated species in bovine pneumonia caused by mycoplasmas (4).

Diagnosis of the respiratory disease is often difficult because significant clinical symptoms cannot be observed in the early period. In the late period, it causes heavy economic losses due to loss of body condition after infection, regression in growth, pneumonia and secondary infections (5).

Isolation of *M. bovis* from the lungs of cattle with pneumonia is often difficult and requires good experience. Cultural isolation may show a wide species distribution and therefore sometimes *M. bovis* cannot be detected. On the other hand, the use of PCR method for molecular detection of *M. bovis* is critical for early diagnosis and treatment.

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Various PCR techniques are used in the molecular diagnosis of the agent. Therefore, PCR-based molecular methods can be more advantageous in such analyzes and false negative rates are further reduced (6-11).

Kleinschmidt et al. (11) reported that *M. bovis* was detected by PCR method at a rate of 25-33% in cattle with respiratory system disease in countries such as England, Spain, Denmark, France, Switzerland, Germany and Israel.

Due to the isolation and identification problems of mycoplasma species by cultural methods, a rapid diagnosis cannot be made in the early stages of the disease. Despite this fact, it is seen that there are few molecular epidemiological studies on this subject in Türkiye (12-18).

Sayın et al. (19) determined that, the presence of respiratory system infections due to mycoplasma were in 17 (80.9%) of 21 different dairy farms located in 7 geographical regions in Türkiye. In their study, *M. bovis* was isolated and identified by cultural methods while 149 (87.6%) of 172 clinical samples found to be positive for *Mycoplasma* spp. by PCR.

Karahan et al. (20) were investigated *M. bovis* in different clinical samples that were taken from 148 animals located in three different farms in the Eastern Anatolia Region. As a result, all 3 lung samples, 23.5% of 51 nasal swabs and 21.1% of 90 milk samples were found to be positive by cultural and PCR analyses.

It has been reported that (13) they observed macroscopic pneumonia lesions in 100 (3.89%) of 2565 lung samples taken from bovine in the slaughterhouse. As a result of the PCR analysis, they stated that *M. bovis, M. dispar* and *M. bovinrhinis* were identified in 19 of the samples

The prevalence of *M. bovis* was determined as 7.5% in calf pneumonia in the Trakya and Marmara Region by bacteriologic culture analysis (21). In another study conducted for the diagnosis of *M. bovis* in bovine lung samples in Erzurum province by PCR method, 36% positivity was detected, while this rate was reported to be 4% in Kars province (13).

In some studies, it was stated that obtaining the collected samples, especially from groups with clinical signs of respiratory system diseases and with herd problems, was effective in the high rate of positivity for *M. bovis* (22, 23).

It is possible to isolate the agent from the lesioned tissues of the lung, lymph nodes and pleural fluid by cultural methods (8). In some studies, for the diagnosis of *M. bovis* by PCR, it was reported that positivity was higher especially in cases of pus-necrotic bronchopneumonia (13, 14, 24, 25).

In this study, it was aimed to investigate the presence and prevalence of *M. bovis* by real time PCR method in lung necropsy samples with pneumonia taken from cattle in South region of Türkiye.

Material and Methods

Ethical Statement: Ethical approval for this study was obtained from Ministry of Agriculture and Forestry, Adana Veterinary Control Institute Animal Experiment Local Ethics Committee (13.11.2017 / 11-3847).

Animal Samples: In this study, 100 lung tissue samples taken from cattle with pneumonia and brought to Adana Veterinary Control Institute were used in 2017 and 2018. Two samples were taken from each lung material which brought to the laboratory. One was stored at -80ºC until examined for detection of *M. bovis* by real time PCR. Another sample was examined by culture method to investigate the presence of other pathogens.

Cultural Analysis: For the isolation of respiratory tract pathogens other than *M. bovis*, the samples were inoculated on to Blood Agar Base (Merck-110886, Germany) supplemented with 5% sheep blood and Mac Conkey Agar (Merck-105465, Germany) and incubated for 24-48 hours at 37ºC. Growing colonies were firstly subjected to Gram staining, hemolysis, and growth test on Mac Conkey Agar. Finally, preliminary identified colonies were identified using the ID panel by the VITEK® 2 Compact (BIOMÉRIEUX-France) instrument.

DNA Isolation: In order to homogenize the lung tissue samples to be examined, 25 mg were taken and placed in MagNA Lyser Green Beads tubes, and 800 µL of 0.9% saline water was added. Then, it was homogenized for 60 seconds at 6000 rpm in a MagNA Lyser (Roche Diagnostics - USA) device and then kept in the Aluminum Cooling Block (Scienfocus lab - USA) for 3-5 minutes. The samples were centrifuged at 8000 rpm for 5 minutes and supernatant was taken into Eppendorf tubes to be used in the tests.

DNA isolations of the samples were performed in the QIAcube (Qiagen, Germany) fully automatic nucleic acid isolation device. QIAamp DNA Mini Kit (Qiagen, UK) was used for DNA isolation and it was performed automatically by the device according to the protocol recommended by the company. The obtained DNA samples were stored in a deep freezer at -20ºC (17, 27).

Real Time PCR: For the amplification of *M. bovis* specific membrane protein gene region by real time PCR, *M. bovis* specific primers were used according to Foddai et al. (27) previously reported (Table 1). Preferred primers amplify a 447 bp gene region.

Commercial Standard real time PCR Detection Kit for *M. bovis* (Primer Design, GENESIG® UK) was used (Table 2) to detect *M. bovis* in DNA samples by real time PCR. The protocol was applied as recommended (Table 3).

Table 1. Oligo sequence used in the study

Target gene	Oligonucleotide sequence (5'-3')	Amplicon Length
mb-mp1	F: TAT TGG ATC AAC TGC TGG AT' R: AGA TGC TCC ACT TAT CTT AG	447 bp

The results in the real time PCR device were evaluated by comparing them with the positive (logarithmic curve) and negative control (solid line). The positive/negative result of a sample was determined by the ct value. If the cycle threshold (ct) value is <30, it is considered positive, if the ct value is between 30-35 and there is a logarithmic curve, it is considered weakly positive, and if the ct value is >35 and the amplification curve has a linear structure, it is accepted as negative.

Table 2. Mastermix components used in the study

Component	Volume (µL)
PrecisionPLUS 2X qPCR Master Mix	10
M.bovis primer/probe mix	1
Internal extraction control primer/probe mix	1
DNase/RNase free water	3
Final volume	15

Table 3. Amplification protocol

Step	Temp (°C)	Time	Cycling
Enzyme Activation	95	2 minutes	1
Denaturation	95	10 seconds	
Annealimg	60	60 seconds	50
Elongation	72	60 seconds	
Final Elongation	72	2 minutes	1

Results

Macroscopic Examinations: The lung samples were macroscopically examined in detail by opening the lumen of the bronchi and bronchioles. On the outer and cross-sectional surfaces of the lobes, diffuse areas of necrosis were observed mostly in the appearance of a typical mottled marble landscape, as well as gray-cream colored partly hard consistency and nodular character. It was determined that the lesioned lung tissues had a harder consistency than the normal lung tissue, and necrosis particularly affected the lung regions around the bronchi and bronchioles. On the cross-sectional surfaces of some lesioned areas, the presence of a necrotic-purulent exaudate surrounded by a connective tissue capsule and the presence of coagulation necrosis in some samples were observed (Figure 1). In some samples, it was observed that the lungs were affected at the lobar level, and these regions were viscous and voluminous (Figure 2).

Real Time PCR Test Results: In the examination performed with real time PCR, 52 of 100 samples were found positive for *M. bovis* (Table 4, Figure 3). Of the positive samples, 46 were obtained from adult cattle, 6 from calves.

Fortyeight of the samples were found negative for *M. bovis* by real time PCR. Of these samples, 45 were adult cattle and 3 were calves (Table 4).

Figure 1. Lesioned lung tissue with purulent-necrotic bronchopneumonia

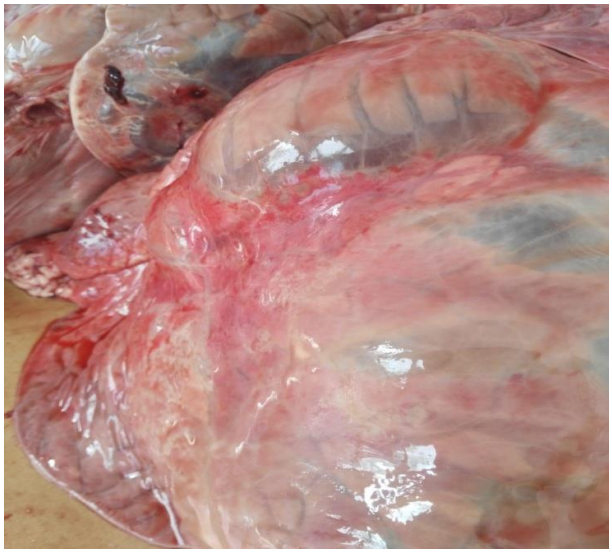

Figure 2. Marble appearance of lung tissue with fibrinous necrotic bronchopneumonia

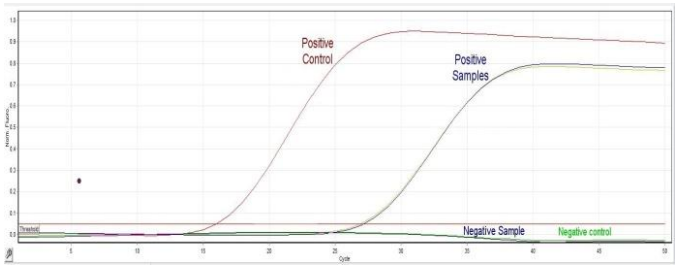

Figure 3. Real time PCR test results

Table 4. Real time PCR analysis results

***Mycoplasma bovis* real time PCR analysis**	**Cattle**	**Calve**	**Total**
Positive	46	6	52
Negative	45	3	48
Total	**91**	**9**	**100**

Bacteriological Culture Analysis Results: Bacterial agents were isolated from 50 of 100 Lung samples examined in the study. Nine of culture positive lung samples were contained more than one bacterium and a single agent was isolated from 41 of them.

No other bacterial agents could be isolated by bacteriological culture in 29 of 52 samples that were found to be *M. bovis* positive by real time PCR method. In 23 of the samples, different types and numbers of bacteria were grown, while 2 different bacteria were detected in 3 of them. In the other 20, a single bacterial species was isolated and identified (Table 5, 6).

M. bovis positive samples by real time PCR were also analyzed by bacteriological culture method. According to the results obtained, *Escherichia coli*, *Pasteurella multocida, Mannheimia haemolytica, Klebsiella pneumonia, Sphingomonas paucimobilis* and *Staphylococcus* spp. were isolated in different rate and identified (Table 5).

Bacterial agent was isolated by culture method from 27 of 48 lung samples that were negative for *M. bovis* by real time PCR method, but no bacterial agent could be isolated from 21. While a single agent was isolated in 21 of the culture-positive samples, more than one agent was isolated in 6 of them.

E. coli from 10, *P. multocida* from 6, *K. pneumonia* from 6, *S. paucimobilis* from 3, *M. haemolytica* from 1 and *S. epidermidis* from 1 of *M. bovis* negative samples by real time PCR were identified by culture (Table 6).

Table 5. Distribution of other bacterial species in samples found positive for *M. bovis* by real time PCR

Bacteria isolated and identified by culture method	*M. bovis* real time PCR positive		Total (%)
	Cattle	Calve	
M. haemolytica	3	0	3 (5.77)
P. multocida	5	0	5 (9.62)
K. pneumonia	1	0	1 (1.92)
K. pneumonia and *E. coli*	2	0	2 (3.85)
S. paucimobilis	2	0	2 (3.85)
S. paucimobilis and *E. coli*	1	0	1 (1.92)
Staphylococcus spp.	1	0	1 (1.92)
E. coli	7	1	8 (15.38)
Culture positive	24	5	29 (55.77)
Total	**46**	**6**	**52 (100)**

Table 6. Distribution of other bacterial species in samples found negative for *M. bovis* by real time PCR

Bacteria isolated and identified by culture method	*M. bovis* real time PCR negative		Total (%)
	Cattle	Calve	
M. haemolytica	1	0	1 (2.08)
P. multocida	5	0	5 (10.42)
P. multocida and *S. paucimobilis*	1	0	1 (2.08)
K. pneumonia	2	0	2 (4.17)
K. pneumonia and *E. coli*	2	0	2 (4.17)
K. pneumonia and *P. multocida*	2	0	2 (4.17)
S. paucimobilis	2	0	2 (4.17)
S. paucimobilis and *E. coli*	1	0	1 (2.08)
S. epidermidis	1	0	1 (2.08)
E. coli	10	0	10 (20.83)
Culture negative	18	3	21 (43.75)
Total	**45**	**3**	**48 (100)**

Discussion

Pneumonia are one of the important problems in cattle breeding. Mycoplasmas are detected at a significant rate among the agents of pneumonia, and the most common among them is *M. bovis*. Pneumonia cases caused by mycoplasmas have been reported for different years (14, 18, 28).

In studies conducted in various countries, differences in *M bovis* isolation rates from pneumonia cases were observed. It has been reported that *M. bovis* is isolated in 11.8% in Hungary (29), 13-23% in Ireland (30, 31), 25% in Italy (32), 30% in France (7) and 91% in Canada (33).

In various studies conducted in different regions of Türkiye, it is reported that the isolation rate of *M. bovis* varies between 4% and 81.3% (21, 28, 34-36).

In this study, a total of 100 bovine lung samples were examined by molecular and cultural methods. Out of 52 (52%) examined samples were found positive for *M. bovis* by real time PCR. In studies on the prevalence of *M. bovis* in various countries (11.8-91%) and Türkiye (4-81.3%), it has been reported that it can be isolated at different rates. The results obtained in this study are similar to the isolation rates obtained in both Türkiye and other countries. In particular, isolation by molecular or bacteriological culture method may cause proportional differences since the sensitivity of molecular methods is higher than the bacteriological culture method.

Since the host defense system is weakened in *M. bovis* infections, a predisposing situation may occur in terms of secondary pathogens. In studies of mixed infections, Arcangioli et al. (37) stated that *M. bovis* was detected in the early stage of pneumonia and *M. bovis* may be the primary or predisposing factor in the formation of bovine respiratory disease outbreaks.

Haines et al. (26) reported that they detected *M. bovis* antigen by immunohistochemical method in the 35 lungs and the 22 joints of 49 cattle with chronic pneumonia that did not respond to treatments. They reported that out of *M. bovis* antigen positive cases, 39% were BVDV, 10% *H. somni*, 20% *M. haemolytica*, and 12% vere found to be both BVDV and *M. haemolytica* antigen positive.

Bacterial agents isolated together with *M. bovis* include *M. haemolytica, P. multocida, Trueperella pyogenes* and less frequently *H. somni*, and viral agents such as bovine respiratory syncytial virus, BHV-1 and BPIV-3. agents are reported (38). Brice et al. (30) also stated that they isolated 20.5% *M. haemolytica*, 9.06% *T. pyogenes* and 8.36% *P. multocida* in 287 animals with

positive *M. bo*vis. Şahin (34) isolated various mycoplasma species (6 *M. bovis*, 4 *M. bovirhinis* and 2 *M. arginine*) from 12 of 109 cattle lungs with pneumonia. Seven of them were *P. multocida*, *M. haemolytica* and *Staphylococcus* spp. reported positive. Byrne et al. (31) reported that in 66% of the *M. bovis* positive cases they detected in Ireland, they also detected viral agents such as BHV-1 and BPIV-3, as well as other bacteria such as *P. multocida* and *M. haemolytica*.

Various bacterial agents were detected by culture method in 50 of 100 lung samples examined in this study. While 9 of the culture positive samples contained more than one agent, only one agent was isolated from 41 of them. Different species and numbers of bacteria were detected in 23 of 52 samples found positive for *M. bovis* by real time PCR. While 2 different bacteria were detected in 3 of them, a single bacterium was isolated and identified in the other 20. In this study, bacterial culture positivity was detected in 23 of the samples found positive for *M. bovis* by real time PCR. Out of 23 *M. bovis* positive samples, *E. coli* from 8, *P. multocida* from 5, *M. haemolytica* from 3, *K. pneumonia* from 3, *S. paucimobilis* from 3, and *Staphylococcus* spp. from 1 sample were isolated and identified. Among these samples, *E. coli* was also isolated and identified in 2 *K. pneumonia* and 1 *S. paucimobilis* positive samples. Various bacterial agents were isolated by culture method from 27 of 48 lung samples that were negative for *M. bovis* by real time PCR. A single agent was isolated in 21 of the samples that were positive for bacteriological culture. More than one agent was isolated in 6 of them.

In studies on the subject, it was observed that other pathogens isolated from *M. bovis* positive animals showed similarities with the isolated strains in this study. As can be seen, suppression of the immune system in pneumonia cases and other predisposing factors that develop in the tissues provide an environment for the inclusion of secondary factors. The existence of multifactorial pneumonia cases observed in other studies was once again demonstrated in this study.

The results obtained in this study show that *M. bovis* is a common and important (52%) pneumonia agent in southern Türkiye. In the study, different types and numbers of bacteria were isolated in 44.2% of the samples that were positive for *M. bovis* by real time PCR, while 2 different bacteria were isolated and identified in 3 of them, and a single bacterium was isolated and identified in the other 20. In such studies, it is very difficult to determine the primary factor. On the other hand, the inability to detect different agents in *M. bovis* positive samples increases the possibility of *M. bovis* being the primary agent.

It was concluded that the high rate of *M. bovis* positivity in the study was due to the real time PCR method, which is shown as a reference method among diagnostic methods in the literature and used in this study.

As a result, pneumonia cases are frequently seen in cattle in Türkiye as well as all over the world and cause significant economic losses in many regions. For this reason, pneumonia cases are an important problem that should be carefully considered. In the prevention of disease, business management errors, negative care conditions and stress factors should not be ignored in terms of herd management.

References

1. Gül Y, Dabak M, Kalender H, Kızıl O, İssi M. Enzootik pnömonili dana ve kuzularda amoksisilinle tedavi denemeleri. Bültendif 1999; 12: 12-15.
2. Özçelik M, İssi M, Gül Y, ve ark. Bakteriyel pnömonili besi sığırlarında oluşan serbest radikal hasarının antioksidan aktivite ve bazı mineral maddeler üzerine etkisi. Erciyes Univ Vet Fak Derg 2014; 11: 111-116.
3. İssi M, Eröksüz Y, Öngör H, ve ark. Enzootik pnömoni semptomları görülen bir besi sığırı işletmesinde Mycoplasma bovis enfeksiyonu. Atatürk Üniversitesi Vet Bil Derg 2015; 10: 39-45.
4. Constable PD, Hinchcliff KW, Done SH, Grünberg W. Veterinary Medicine. 11th Edition, Missouri: W.B. Saunders, Elsevier, 2017.
5. Güneş V. Buzağı Solunum Sistemi Hastalıkları. Lalahan Hay Araşt Enst Derg 2018; 58: 35-40.
6. Ter Laak EA, Noordergraaf JH, Boomsluiter E. The nazal mycoplasmal flora of healthy calves and cows. J Vet Med B 1992; 39: 610-616.
7. Grand DL, Calavas D, Brank M, et al. Serological prevalence of Mycoplasma bovis infection in suckling beef cattle in France. Vet Rec 2002; 150: 268-273.
8. Thomas A, Ball H, Dizier I, et al. Linden Isolation of mycoplasma species from the lower respiratory tract of healthy cattle and cattle with respiratory disease in Belgium. Vet Rec 2002; 151: 472-476.
9. Hirose K, Kobayashi H, Ito N, et al. Isolation of mycoplasmas from nasal swabs of calves affected with respiratory diseases and antimicrobial susceptibility of their isolates. J Vet Med B 2003; 50: 347-351.
10. Rifatbegovic M, Assuncao P, Poveda JB, Pasic S. Isolation of Mycoplasma bovis from the respiratory tract of cattle in Bosnia and Herzegovina. Vet Rec 2007; 160: 484-485.
11. Kleinschmidt S, Spergser J, Rosengarten R, Hewicker Trautwein M. Long-term survival of Mycoplasma bovis in necrotic lesions and in phagocytic cells as demonstrated by transmission and immunogold electron microscopy in lung tissue from experimentally infected calves. Vet Microbiol 2013; 162: 949-953.
12. Nicholas RAJ, Ayling RD, Stipkovits LP. An experimental vaccine for calf pneumonia caused by Mycoplasma bovis: Clinical, cultural, serological and pathological findings, Vaccine 2002; 20: 3569-3575.
13. Özen H, Karaman M, Şahin M, Özcan K. PCR Detection of Mycoplasma bovis, M. dispar, M. bovirhinis and M. mycoides subsp. mycoides (small colony type) and Investigations of Pathological Findings in Pneumonic Cattle. Kafkas Univ Vet Fak Derg 2009; 15: 125-133.

14. Yılmaz R. Sığırlarda Mycoplasma bovis Pnömonilerinde Histopatolojik ve İmmunohistokimyasal Bulgular. Doktora tezi. Bursa: Bursa Uludağ Üniversitesi, Sağlık Bilimleri Enstitüsü, 2009.

15. Rosetti BC, Frey J, Pilo P. Direct detection of Mycoplasma bovis in milk and tissue samples by real-time PCR. Mol Cell Probes 2010; 24: 321-323.

16. Arcangioli MA, Aslan H, Tardy F, Pourmarat F, Le Grand D. The use of pulsed- field gel electrophoresis to investigate the epidemiology of Mycoplasma bovis in French calf feedlots. The Vet J 2012; 192: 96-100.

17. Adamu JY, Wawegama NK, Browning GF, Markham PF. Membrane proteins of Mycoplasma bovis and their role in pathogenesis. Res Vet Sci 2013; 95: 321-325.

18. Altun S. Pnömonili Sığır Akciğerlerinde Mycoplasma bovis Enfeksiyonunun Patolojik ve Moleküler Yöntemlerle Araştırılması. Doktora tezi. Erzurum: Atatürk Üniversitesi; Sağlık Bilimleri Enstitüsü, 2015.

19. Sayın Z, Sakmanoğlu A, Uçan US, et al. Mycoplasma infections in dairy cattle farms in Turkey. Turk J Vet Anim Sci 2016; 40: 569-574.

20. Karahan M, Kalin R, Atil E, Çetinkaya B. Detection of Mycoplasma bovis in cattle with mastitis and respiratory problems in eastern Turkey. Vet Rec 2010; 166: 827-829.

21. Erdağ O, Erdoğan G, Türkaslan J, Gürel A. Buzağı ve dana pneumonilerinde Mycoplasma ve bakteriyel etkenlerin izolasyon, identifikasyon ve antibiyotik duyarlılıkları. Animal Information 1995; 112: 115-119.

22. Soehnlen MK, Kunze ME, Karunathilake E, et al. In vitro antimicrobial inhibition of Mycoplasma bovis isolates submitted to the Pennsylvania Animal Diagnostic Laboratory using flow cytometry and broth microdilution method. J Vet Diagn Invest 2011; 23: 547-551.

23. Akan M, Babacan O, Torun E, Müştak HK, Öncel T. Diagnosis of Mycoplasma bovis Infection in Cattle by ELISA and PCR. Kafkas Univ Vet Fak Derg 2014; 20: 249-252.

24. Maeda T, Shibahara T, Kimura K, et al. Mycoplasma bovis-associated suppurative otitis media and pneumonia in bull calves. J Comp Pathol 2003; 129: 100-110.

25. Gabinaitiene A, Sıugzdaıte J, Zilinskas H, Siugzda R, Petkevicius S. Mycoplasma bovis and bacterial pathogens in the bovine respiratory tract. Vet Med 2011; 56: 28-34.

26. Haines DM, Martin KM, Clark EG, Jim GK, Janzen ED. The immunohistochemical detection of Mycoplasma bovis and bovine viral diarrhea virus in tissues of feedlot cattle with chronic, unresponsive respiratory disease and/or arthritis. Can Vet J 2001; 42: 857-860.

27. Foddai A, Idini G, Fusco M, et al. Rapid differential diagnosis of Mycoplasma agalactiae and Mycoplasma bovis based on a multiplex-PCR and a PCR-RFLP. Mol Cell Probe 2005; 19: 207-212.

28. Önat K. Sığır pnömonilerinde Mycoplasma bovis varlığının kültür ve ELISA yöntemleri ile araştırılması. Doktora tezi. Bursa: Bursa Uludağ Üniversitesi, Sağlık Bilimleri Enstitüsü, 2011.

29. Tenk M, Stipkovits L, Hufnagel L. Examination of the role of Mycoplasma bovis in bovine pneumonia and a mathematical model for its evaluation. Acta Vet Hung 2004; 52: 445-456.

30. Brice N, Finlay D, Bryson DG, Henderson J, Mcconnell W, Ball HJ. Isolation of Mycoplasma bovis from cattle in Northern Ireland, 1995 to 1998. Vet Rec 2000; 146: 643-644.

31. Byrne WJ, Mccormack R, Brice N, Egan J, Markey B, Ball HJ. Isolation of Mycoplasma bovis from bovine clinical samples in the Republic of Ireland. Vet Rec 2001; 148: 331-333.

32. Radelli E, Luini M, Loria GR, Nicholas RAJ, Scanziani E. Bacteriological, serological, pathological and immunohistochemical studies of Mycoplasma bovis respiratory infection in veal calves adult cattle at slaughter. Res Vet Sci 2008; 85: 282-290.

33. Shahriar FM, Clark EG, Janzen E, West K, Wobeser G. Coinfection with bovine viral diarrhea virus and Mycoplasma bovis in feedlot cattle with chronic pneumonia. Can Vet J 2002; 43: 863-868.

34. Şahin M. Kars yöresinde sığır pnömonilerinden Mikoplazmaların izolasyonu, identifikasyonu ve antibiyotiklere olan duyarlılıklarının belirlenmesi. Etlik Vet Mikrobiyol Derg 1997; 9: 71-89.

35. Özdemir Ü, Türkyılmaz MA. Buzağılarda önemli pnömoni etkenlerinden Mycoplasma bovis'in izolasyonu ve identifikasyonu. VIII. Ulusal Veteriner Mikrobiyoloji Kongresi, Van, 2008: 82.

36. Çetinkaya B, Karahan M, Kalın R, Atıl E. Türkiye'nin doğusundaki ruminant mikoplazmalarının biyoçeşitliliği: Aşı ve kontrol stratejileri için uygulamalar. IX. Ulusal Veteriner Mikrobiyoloji Kongresi (Uluslararası katılımlı), Kongre özet kitabı, 2010: 4-7.

37. Arcangioli MA, Duet A, Meyer G, et al. The role of Mycoplasma bovis in bovine respiratory diseae outbreaks in veal calf feedlots, The Vet J 2008; 177: 89-93.

38. Nicholas R, Ayling R, McAuliffe L. Mycoplasma diseases of ruminants. Cambridge, MA; Wallingford, UK: CABI; 2008.132-168.