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Mycoplasma Infection in the Lungs of Cattle: The First Identification of *Mycoplasma dispar* in Iran

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Abstract

BACKGROUND: Members of the genus *Mycoplasma* are known as pathogens causing respiratory disease in cattle worldwide.

OBJECTIVES: The present study aimed to investigate mycoplasmal infection in the lung tissue of cattle slaughtered in Hamadan industrial abattoir, Iran, using molecular and histopathological methods.

METHODS: A total of 108 tissue samples were collected from the cranioventral parts of the cattle lung during March 2015-February 2016. The specimens were subjected to a polymerase chain reaction (PCR) and histopathological examinations. The PCR-positive samples were tested subsequently for *Mycoplasma bovis* and *Mycoplasma dispar* using nested PCR assay.

RESULTS: Nine (8.33%) samples contained the DNA of genus *Mycoplasma*, among which, five and one showed the DNA sequences of *M. bovis* and *M. dispar*, respectively. Pathological changes, such as caseonecrotic lesions, interstitial pneumonia, lobar bronchopneumonia, and bronchial atelectasis were observed in 24 (22.22%) tissue samples. All the PCR-positive lungs demonstrated at least one pathological manifestation. However, not every pathognomonic tissue changes were concomitant with the presence of the DNA of *Mycoplasma* spp.

CONCLUSIONS: It could be concluded that *M. bovis* and to a lesser extent *M. dispar* are relatively common in the cattle population of the western part of Iran. Therefore, these pathogens should be taken into consideration whenever respiratory problems are evident in cattle.

KEYWORDS: *Mycoplasma*, *Mycoplasma bovis, Mycoplasma dispar*, Nested PCR, Respiratory disease

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Introduction

Bovine respiratory disease is the most common illness affecting housed cattle worldwide (van Leenen *et al.*, 2019). Several *Mycoplasma* species have been isolated from the bovine respiratory tract, including *Mycoplasma bovis*, *Mycoplasma dispar*, *Mycoplasma bovirhinis*, and *Mycoplasma leachii* (Becker *et al.*, 2015; Spergser *et al.*, 2019). Members of *Mycoplasma*, a genus of class *Mollicutes*, are free-living, self-replicating tiny prokaryotes (Parker *et al.*, 2018) with no cell wall. These agents have the smallest known bacterial genome in the form of a single circular chromosome of double-stranded DNA (Becker *et al.*, 2015).

These bacteria live generally as intracellular parasites close to the nucleus of their hosts (Szacawa *et al.*, 2016).

The organism colonizes mucosal surfaces and produces several diseases, namely pneumonia, arthritis, otitis media, and meningitis, in calves, and mastitis and genital infections in adult cows (Schibrowski *et al.*, 2018). Various factors, such as stress and simultaneous viral and bacterial infections (e.g., syncytial virus and *Pasteurella multocida*) have been reported as the predisposing factors for *Mycoplasma* infection (Bürki *et al.*, 2015; Hay *et al.*, 2014; Wisselink *et al.*, 2019).

M. bovis is the second most pathogenic *Mycoplasma* that causes considerable economic losses in cattle, especially as calf pneumonia (Van Leenen *et al.*, 2019). *M. bovis* has been detected in most countries throughout the world (Nicholas 2011) after the first detection as an etiologic agent of bovine mastitis in the USA (Hale *et al.*, 1962). Similarly, the pathogen has been reported from different parts of Iran (Baharsefat and Yamini, 1968; Ghazaei, 2006; Dabiri *et al.*, 2018; Imandar *et al.*, 2018). *M. dispar* causes exudative bronchitis and pneumonia in calves, especially through the inhalation of contaminated aerosol and materials (Friis, 1980).

The amplification of specific genes by polymerase chain reaction (PCR) provides a fast and reliable way to detect *Mycoplasma* spp., compared to traditional methods, such as bacteriological culture (Hotzel *et al.*, 1996; Chen *et al.*, 2019).

Considering the lack of information on the state of mycoplasmal infection in the cattle population of the region, this study was carried out to detect Mycoplasmal infection in the lung tissue of cattle slaughtered in Hamedan industrial abattoir, Iran using PCR and histopathological examinations.

Materials and Methods

Sample Collection

A total of 108 tissue samples (3×3×1 cm) were collected from the cranioventral regions of the cattle lungs during March 2015-February 2016 in Hamedan industrial abattoir regardless of the age, gender, breed, and origin of animals. The samples were placed in sterile plastic bags inside a cold box and were transported to the research laboratory of the Faculty of Veterinary, Bu-Ali Sina University, Hamadan, Iran. The tissue specimens were immediately cut into two pieces, one of which was placed in 10% neutral buffered formalin and the other piece being stored at -20ºC until further analysis.

DNA Extraction

Approximately 20 mg of each specimen was cut into small pieces aseptically and was utilized for DNA extraction by a commercial DNA extraction kit (Yekta Tajhiz Azma Co., Iran) according to the manufacturer's manual. The purified DNA samples were stored in 1.5 mL microtubes at -70°C until evaluation by PCR. The DNA was also extracted from the *Mycoplasma agalactiae* vaccine (Razi Vaccine and Serum Research Institute, Iran) to be used as a positive control in *Mycoplasma* genus PCR.

Molecular Detection of *Mycoplasma* Genus

The extracted DNA samples were molecularly examined using the specific primers of

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Mycoplasma genus (GPF and MGSO) indicated in Table 1 (Lierz *et al.*, 2007; Van Kuppeveld *et al.*, 1994). The PCR mixture (20 µL) contained a commercial PCR Master Mix (10 µL), GPF primer (0.25 µL), MGSO primer (0.25 µL), target DNA (5 µL), and nuclease-free water (4.5 µL). The amplification settings entailed pre-denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, 72°C for 1 min, and a final extension step at 72°C for 10 min in a thermal cycler (ASTEC Co., Fukuoka, Japan). Positive and negative controls were included in all PCR runs. Finally, 10 µL of the PCR products were electrophoresed on 1.5% agarose gel containing ethidium bromide (0.5 µg/mL) and visualized under the UV light utilizing a UV-transilluminator. A PCR product of 1013 bp in size was indicative of *Mycoplasma* genus microorganisms (Lierz *et al.*, 2007).

Identification of *M. bovis* by Nested PCR

Following the characterization of samples infected with *Mycoplasma*, the positive DNAs were tested for *Mycoplasma* species using nested and conventional PCR for *M. bovis* and *M. dispar*, respectively. As presented in Table 1, the outside PCR reactions used primers PpMB920-1 and PpMB920-2, while the inside one was carried out by primers PpSM5-1 and PpSM5-2 for *M. bovis* (Hotzel *et al.*, 1993; Pinnow *et al.*, 2001).

The nested PCR cycling parameters encompassed an initial denaturation step at 94°C for 11.5 min followed by 35 cycles of 94°C for 30 sec, primer annealing at 48°C for 60 sec, extension at 72°C for 150 sec, and a final step of 72°C for 10 min. A volume of 5 µL at a dilution of 1:100 of the outside PCR product was used as the template DNA in the second PCR reaction. The thermal conditions for the second PCR were almost similar to the outside PCR except for the annealing temperature which was 54°C. The total reaction volume of 25 µL had 12.5 µL of a commercial PCR Master Mix, 0.5 µL of each specific primer for *M. bovis* PCR 1 and 2, target DNA (5 µL), and nuclease-free water (6.5 µL). Afterwards, the PCR products were resolved by the same electrophoresis protocol as described above.

Identification of *M. dispar* by PCR

Samples devoid of the expected DNA for *M. bovis* species were tested for the genomic DNA of *M. dispar* using primers MDF and MDR demonstrated in Table 1 (Marques *et al.*, 2007). The 25 µL PCR mixture included 12.5 µL of a commercial PCR Master Mix, 1 µL of each primer (50 pmol), 5.5 µL target DNA, and 5 µL nuclease-free water. The amplification process was carried out under the following condition: 94°C for 5 min, 94°C for 1 min, 53.6°C for 1 min, and 72°C for 1 min (35 cycles) along with a final cycle at 72°C for 5 min. The same procedure of agarose gel electrophoresis was applied to check PCR products.

Table 1. Primers used for the detection of *Mycoplasma* genus and species in PCR assays.

Primer*	Sequence 5'-3'	Target	Amplicon size (bp)	Reference
GPF MGSO	GCTGGCTGTGTGCCTAATACA TGCACCATCTGTCACTCTGTTAACCTC	*Mycoplasma* genus	1013	Lierz *et al.*, 2007
PpMB920-2 PpMB920-1	TTTTAGCTCTTTTTGAACAAAT GGCTCTCATTAAGAATGTC	*M. bovis*	1911	Hotzel *et al.*, 1993

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Primer*	Sequence 5'-3'	Target	Amplicon size (bp)	Reference
PpSM5-1 PpSM5-2	CCAGCTCACCCTTATACATGAGCGC TGACTCACCATTTAGACCGACTATTTCAC	*M. bovis*	442	Pinnow *et al.*, 2001
MDF MDR	TTAAAGCTCCACCAAAAA GTATCTAAAGCGGACTAAA	*M. dispar*	433	Marques *et al.*, 2007

* All primers were purchased from BioNEER (South Korea).

Sequencing of PCR Products

Because of the unavailability of *M. bovis* and *M. dispar* standard strains as positive controls in PCR reactions, identification of these *Mycoplasma* species was performed by the sequencing of some PCR products (Bioneer, South Korea).

Histopathology

The formalin-fixed samples were dehydrated in ascending ethanol concentrations, cleared in xylene, infiltrated and embedded in paraffin, and sectioned at 5-6 µm thickness using a rotary microtome (Leica RM2255, Germany). The sections were then stained with hematoxylin and eosin (H&E) and were examined independently by a pathologist under a light microscope (Olympus CX41, Japan) equipped with a digital camera (Olympus DP25, Germany).

Results

PCR Assays

As shown in Figure 1, PCR assessment of lung tissue samples for *Mycoplasma* infection revealed that 9 (8.33%) out of 108 samples were contaminated with the DNA of *Mycoplasma* cells. Moreover, all these samples were investigated to determine *Mycoplasma* species. Afterwards, the 442 bp expected DNA fragment was tested in five *Mycoplasma*-positive samples by nested PCR indicating that these samples were infected by *M. bovis*. The results of the last PCR demonstrated that only one DNA sample was infected with *M. dispar* genomic DNA (Figure 1). The identity of the other three samples remained unknown.

Sequencing

The results of sequencing confirmed the identity of two characterized *Mycoplasma* species, namely *M. bovis* and *M. dispar*. The DNA fragment of 442 bp amplified from the *M. bovis* genome in this study showed ~99.5% homology, compared to sequences recorded in the GenBank with the accession number of CP038861.1. On the other hand, the blasting of the PCR product of 433 bp for *M. dispar* indicated ~99% homology with the corresponding segment of the bacterial 16S rRNA genome with an accession number of NR_025182.1.

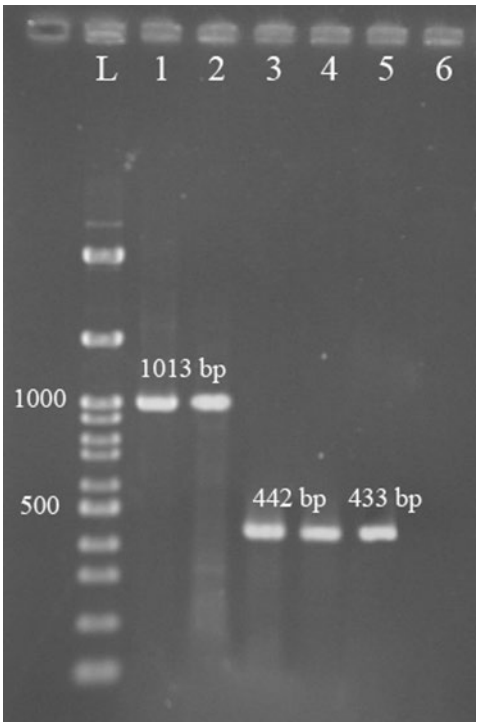

Figure 1. The electrophoresed DNA fragments obtained from PCR reactions to identify *Mycoplasma* genus and species (*M. bovis* and *M. dispar*). Lane L: a 100 bp DNA ladder, lanes 1 and 2: a *Mycoplasma*-positive sample and *M. agalactiae* vaccine as positive control for the genus. Lanes 3 and 4: samples infected with *M. bovis* DNA. Lane 5: the only sample infected with *M. dispar*. Lane 6: a negative sample contained no DNA.

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Histopathology

Microscopic lesions were found in 24 specimens out of 108 (22.22%) pulmonary tissue samples. Caseonecrotic lesions and inflammation sites of various sizes were found in the organ parenchyma of two samples (Figure 2A). Necrotic regions had a concentrated eosinophilic center surrounded by a zone of connective tissue infiltrated by inflammatory cells. Necrotic walls of alveoli and bronchioles were noted in the central area of the necrotic regions. Compressed neighboring alveoli, inflammatory cells, fibroblasts, as well as coagulative necrosis of airways and interstitial tissue were reported around the caseonecrotic regions. In 16 (14.81%) samples, interstitial pneumonia was observed with the thickening of alveolar walls, marked capillary congestion, mononuclear infiltration, and venous congestion of organ stroma (Figure 2B). Lobar bronchopneumonia along with the intense infiltration of neutrophils with fewer macrophages and plasma cells into bronchioles, alveolar ducts, and alveoli were found in three samples (2.77%). Coagulative necrosis of the tissue was noted with bronchial atelectasis, accumulation of purulent exudate in bronchioles and alveoli, and edema and fibrinous accumulation in alveolar ducts and alveolar sacs (Figure 2C). In three samples (2.77%), hyperplasia of lymphatic tissue around bronchioles was found concurrent with the thickening of bronchiolar walls and microvascular congestion (Figure 2D).

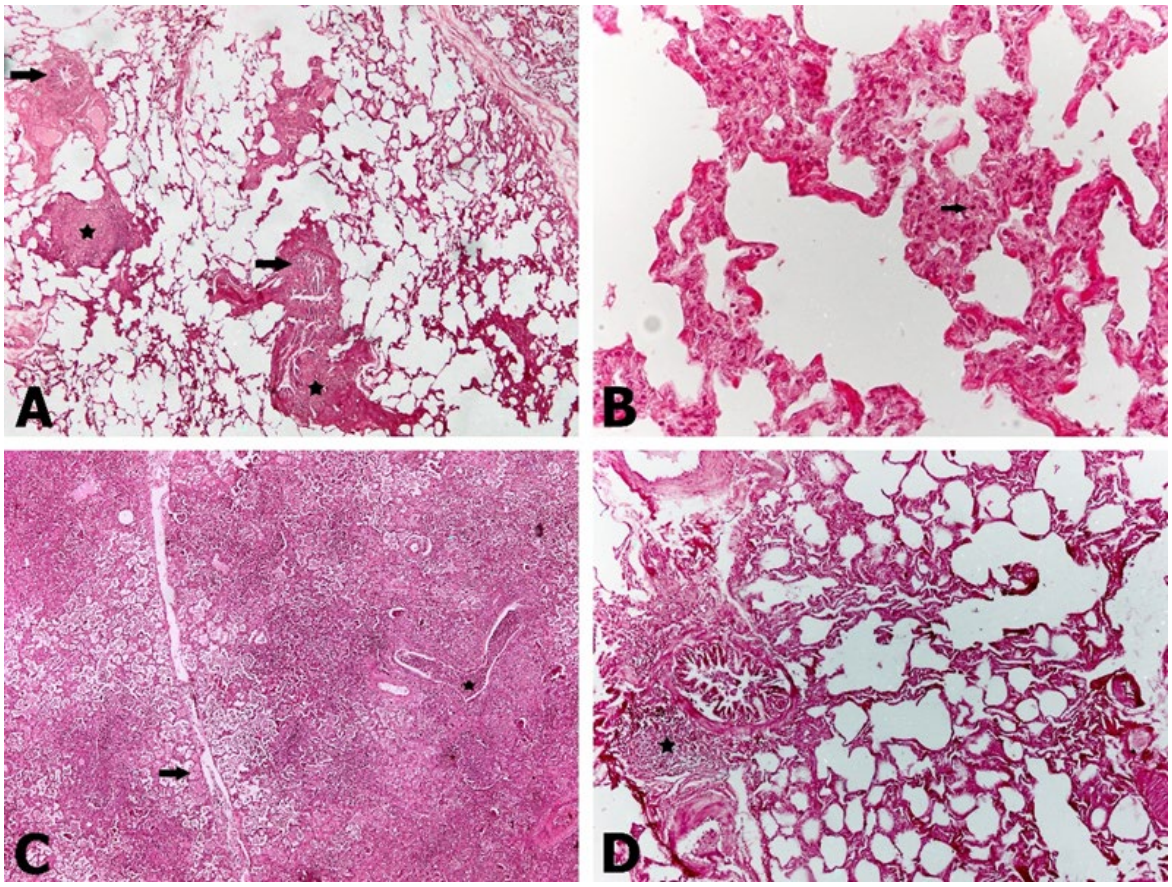

Figure 2. Light micrographs of pulmonary lesions of cattle infected with *Mycoplasma* spp

A. Caseonecrotic lesions in various size and shapes with an eosinophilic homogeneous center in the lung parenchyma (asterisk) nearby bronchioles (arrows). **B**. Interstitial pneumonia with thickening of alveolar walls and infiltration of mononuclear cells (arrow). **C**. Acute lobar bronchopneumonia with inflammatory cell infiltration of bronchioles (asterisk), alveolar ducts and alveoli together with edema and fibrinous exudate in the air sacs (arrow). **D**. Hyperplasia of bronchiolar associated lymphoid tissue along with thickening of the muscular layer of the bronchiole (asterisk).

A, C = x40, B = x400, D = x100

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Hematoxylin and Eosin

Discussion

Mycoplasmas are known to infect cattle worldwide. Several studies on the prevalence of *Mycoplasma* infection in calves have been conducted in Europe, Asia, and North America with results ranging from less than 5% to almost 100% (Becker *et al.*, 2015; Schibrowski *et al.*, 2018; Chen *et al.*, 2019). These organisms have been frequently isolated from the respiratory tract of healthy cattle leading to some speculations about the pathogenicity of the agent (Blackburn *et al.*, 2007). *M. bovis* causes chronic cranioventral bronchopneumonia characterized by caseous necrosis resulting in significant economic losses due to the costs of treatment and laboratory diagnosis, in addition to a decline in dairy and beef cattle production (Fulton, 2009).

In vitro culture of Mycoplasmas is difficult, time-consuming, and expensive often resulting in false-negative results (Waites *et al.*, 2012). A histopathologic examination may show nonspecific lesions, and even, some animals may harbor the bacteria without developing enough pathological changes to exactly be pointed out under the microscope. On the other hand, molecular techniques, such as PCR offer high sensitivity and specificity for the diagnosis of microbial infections in specimens. Therefore, molecular methods are appropriate for detecting mycoplasmas in tissue samples difficult to culture.

Few studies have investigated mycoplasmal infections in cattle in Iran. However, only milk samples were examined in all these researches. Ghazaei (2006) tested 80 milk samples collected from dairy cows with clinical mastitis in the Moghan region of Ardabil, Iran using bacteriological culture and immunoperoxidase methods. The findings indicated that *M. bovis* was isolated from 39 (48.75%) samples (Ghazaei, 2006). In one study in Iran, bacteriological examination of bronchoalveolar lavage fluid resulted in the isolation of *Mycoplasma* from one (14.3%) healthy and four (28.6%) pneumonic calves under 3 months old (Araghi soureh *et al.*, 2007). Imandar *et al.* (2018) performed a study based on bacteriological culture and 16S rRNA PCR on a panel of 328 milk samples collected from cows with clinical symptoms of mastitis from all regions of Iran. Their results indicated that out of 328 samples, 97 (29.57%) cases were infected with the bacteria of genus *Mycoplasma*, among which, 31 (31.97%) samples were identified as *M. bovis* species by PCR assay (Imandar *et al.*, 2018). Dabiri *et al.* (2018) tried to detect *M. bovis* using nested PCR for 104 bulk tank milk samples in Mashhad, Iran. Although *M. bovis* was not identified in any of the specimens, the researchers characterized two *Mycoplasma* species, namely *M. canadense* and *M. yeatsii* by sequencing PCR products (Dabiri *et al.*, 2018).

To our knowledge, the species *M. dispar* was identified in the present study for the first time in Iran. Located in the mountainous region of the country with a cold temperate, Hamedan is one of the main areas of breeding livestock, such as cattle in Iran. However, there is no documented data regarding the infection of cattle with *Mycoplasma* spp. in the region. Furthermore, *M. bovis* and *M. dispar* have not been detected in the lungs of cattle from Iran.

A total of 108 lung tissue samples were subjected to PCR and histopathologic examination for *Mycoplasma* infection. The results showed that nine (8.3%) specimens were positive for genus *Mycoplasma*. Nested PCR revealed that five and one of the positive samples were infected with *M. bovis* and *M. dispar*, respectively. This highlights the importance of *M. bovis* infection in cattle in Iran because over 50% of all identified *Mycoplasma* bacteria belonged to this species. The two-step nested PCR can detect even scarce target DNA molecules virtually as just a few copies with no cross-reactivity with the genomic DNA of other

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organisms. In the neighboring country of Turkey, the PCR examination of tracheal swab samples from cattle aged 6-12 months old and suffering from respiratory problems was positive with a mean of 12.6% (Babacan *et al.*, 2014).

Large-scale epidemiological studies are needed to reveal the prevalence and distribution of *Mycoplasma* spp. particularly *M. bovis* and *M. dispar* in the cattle population of Iran. The results of the current study confirmed that the PCR technique shortens the time needed for identifying *Mycoplasma* in tissue, compared to the conventional bacteriological culture and histopathology methods. The latter one can depict typical and uncharacteristic lesions associated with *Mycoplasma* infection. However, exclusively PCR demonstrated the bacteria in the specimen, and bacteriological culture methods may not always successfully isolate these hard-to-culture bacteria. Furthermore, our results highlighted the need for applying appropriate preventive and control measures, such as implementing hygienic cattle farming and developing effective vaccines against *Mycoplasma* infection.

Based on the results of this study, it can be concluded that pulmonary infections by *M. bovis* and with a lesser grade *M. dispar* are present in the cattle population of west of Iran. As a result, this pathogen should be taken into consideration in case of respiratory problems in cattle. More investigations are required on the etiologic agents of respiratory conditions of cattle in the region.

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Conflict of Interest

The authors declare no conflict of interest.

References

Araghi soureh, A., Mokhber dezfouli, M.R., Afshari, G.R., Nadalian, M.G., Zahraei salehi, M.T., Rezakhani, A., *et al.* (2007). Bronchoalveolar lavage and bacteriological findings in calf pneumonia. *J Vet Res*, 62, 87-92.

Babacan, O., Akan, M., Torun, E., Müştak, H., öncel, T. (2014). Diagnosis of *Mycoplasma bovis* infection in cattle by ELISA and PCR. *Kafkas Univ Vet Fak Derg*, 20, 249-252. [DOI:10.9775/kvfd.2013.9959]

Baharsefat, M., Yamini, B. (1968). Animal mycoplasmosis in Iran, *Arch Inst Razi*, 20: 39-42.

Becker, C.A., Thibault, F.M., Arcangioli, M.A., Tardy, F. (2015). Loss of diversity within *Mycoplasma bovis* isolates collected in France from bovines with respiratory diseases over the last 35 years. *Infect Genet Evol*, 33, 118-126. [DOI:10.1016/j.meegid.2015.04.019] [PMID]

Blackburn, P., Brooks, C., McConnell, W., Ball, H.J. (2007). Isolation of *Mycoplasma bovis* from cattle in Northern Ireland from 1999 to 2005. *Vet Rec*, 161, 452-453. [DOI:10.1136/vr.161.13.452] [PMID]

Bürki, S., Frey, J., Pilo, P. (2015). Virulence, persistence and dissemination of *Mycoplasma bovis*. *Vet Microbiol*, 179, 15-22. [DOI:10.1016/j.vetmic.2015.02.024] [PMID]

Chen, S., Hao, H., Yan, X., Liu, Y., Chu, Y. (2019). Genome-wide analysis of *Mycoplasma dispar* provides insights into putative virulence factors and phylogenetic relationships. G3: *Genes, Genomes, Genetics*, 9, 317-325. [DOI:10.1534/g3.118.200941]

Dabiri, M., Mirshokraei, P., Rad, M., Khoramian, B. (2018). Detection of *Mycoplasma bovis* in bulk tank milk samples by nested PCR in Mashhad, *Iran J Vet Sci Technol*, 9, 28-32.

Friis, N.F. (1980). Mycoplasma dispar as a causative agent in pneumonia of calves. *Acta Vet Scand*, 21, 34-42. [DOI:10.1186/BF03546898] [PMID]

www.SID.ir

Fulton, R.W. (2009). Bovine respiratory disease research (1983-2009). *Anim Health Res Rev*, 10, 131-139. [DOI:10.1017/S1466252309990172X] [PMID]

Ghazaei, C. (2006). Mycoplasmal mastitis in dairy cows in the Moghan region of Ardabil State, Iran. *J S Afr Vet Assoc*, 77, 222-223. [DOI:10.4102/jsava.v77i4.382] [PMID]

Hale, H. H., Helmboldt, C. F., Plastridge, W. N., Stula E. F. (1962). Bovine mastitis caused by a *Mycoplasma* species. *Cornell Vet*, 52: 582-91.

Hay, K. E., Barnes, T. S., Morton, J. M. M., Clements, A. C. A., Mahony, T. J. (2014). Risk factors for bovine respiratory disease in Australian feedlot cattle: Use of a causal diagraminformed approach to estimate effects of animal mixing and movements before feedlot entry. *Prev Vet Med*, 117, 160-169. [DOI:10.1016/j.prevetmed.2014.07.001] [PMID]

Hotzel, H., Demuth, B., Sachse, K., Pflitsch, A., Pfutzner, H. (1993). Detection of *Mycoplasma bovis* using in vitro deoxyribonucleic acid amplification. *Rev Sci Tech*, 12, 581-591. [DOI:10.20506/rst.12.2.700] [PMID]

Hotzel, H., Sachse, K., Pfutzner, H. (1996). Rapid detection of *Mycoplasma bovis* in milk samples and nasal swabs using the polymerase chain reaction. *J Appl Bacteriol*, 80, 505-510. [DOI:10.1111/j.1365-2672.1996.tb03249.x] [PMID]

Imandar, M., Pourbakhsh, S., Jamshidian, M., Zahraei salehi, T. (2018). Isolation, identification and molecular characterization of *Mycoplasma bovis* in mastitic dairy cattle by PCR and culture methods. *J Hell Vet Med Soc*, 69, 815-822. [DOI:10.12681/jhvms.16433]

Lierz, M., Hagen, N., Harcourt-Brown, N., Hernandez-Divers, S.J., Luschow, D., Hafez, H.M. (2007). Prevalence of mycoplasmas in eggs from birds of prey using culture and a genus-specific mycoplasma polymerase chain reaction. *Avian Pathol*, 36, 145-150. [DOI:10.1080/03079450701213347] [PMID]

Marques, L.M., Buzinhani, M., Yamaguti, M., Oliveira, R.C., Ferreira, J.B., Mettifogo, E., et al. (2007). Use of a polymerase chain reaction for detection of *Mycoplasma dispar* in the nasal mucus of calves. *J Vet Diagn Invest*, 19, 103-106. [DOI:10.1177/104063870701900118] [PMID]

Nicholas, R. A. (2011). Bovine mycoplasmosis: silent and deadly. *Vet Rec*, 168: 459-62. [DOI:10.1136/vr.d2468] [PMID]

Parker, A.M., Sheehy, P.A., Hazelton, M.S., Bosward, K.L. and House, J.K. (2018). A review of mycoplasma diagnostics in cattle. *J Vet Internal Med*, 32, 1241-1252. [DOI:10.1111/jvim.15135] [PMID] [PMCID]

Pinnow, C. C., Butler, J. A., Sachse, K., Hotzel, H., Timms, L.L., Rosenbusch, R. F. (2001). Detection of Mycoplasma bovis in preservative-treated field milk samples. *J Dairy Sci*, 84, 1640-1645. [DOI:10.3168/jds.S0022-0302(01)74599-7]

Schibrowski, M. L., Gibson, J. S., Hay, K. E., Mahony, T. J. and Barnes, T.S. (2018). *Mycoplasma bovis* and bovine respiratory disease: A risk factor study in Australian feeder cattle. *Prev Vet Med*, 157, 152-161. [DOI:10.1016/j.prevetmed.2018.06.005] [PMID]

Spergser, J., Hess, C., Loncaric, I. and Ramírez, A.S. (2019). MALDI-ToF mass spectrometry is a superior diagnostic tool for the identification and differentiation of mycoplasmas isolated from animals. *J Clin Microbiol*, JCM-00316. [DOI:10.1128/JCM.00316-19] [PMID] [PMCID]

Szacawa, E., Szymańska-Czerwińska, M., Niemczuk, K., Dudek, K., Bednarek, D., Ayling, R. D. (2016). Comparison of serological, molecular and cultural diagnostic methods for the detection of *Mycoplasma bovis* infections in cattle. *Anim Sci Pap Rep*, 34, 351-359.

van Kuppeveld, F. J., Johansson, K.E., Galama, J. M., Kissing, J., Bolske, G., van der Logt, J. T., *et al.* (1994). Detection of mycoplasma contamination in cell cultures by a mycoplasma group-specific PCR. *Appl Environ Microbiol*, 60, 149-152. [DOI:10.1128/AEM.60.1.149-152.1994] [PMID] [PMCID]

van Leenen, K., Van Driessche, L., De Cremer, L., Gille, L., Masmeijer, C., Boyen, F., *et al.* (2019). Factors associated with lung cytology as obtained by non-endoscopic broncho-alveolar lavage in group-housed calves. *BMC Vet Res*, 15, 167. [DOI:10.1186/s12917-019-1921-x] [PMID] [PMCID]

Waites, K.B., Xiao, L., Paralanov, V., Viscardi,

Archive of SID

R.M., Glass, J.I. (2012). Molecular methods for the detection of Mycoplasma and ureaplasma infections in humans: In: the 2011 William Beaumont Hospital Symposium on molecular pathology. *J Mol Diagn*, 14, 437-450. [DOI:10.1016/j.jmoldx.2012.06.001] [PMID] [PMCID]

Wisselink, H.J., Smid, B., Plater, J., Ridley, A., Andersson, A.M., Aspán, A., et al. (2019). A European interlaboratory trial to evaluate the performance of different PCR methods for *Mycoplasma bovis* diagnosis. *BMC Vet Res*, 15, 86. [DOI:10.1186/s12917-019-1819-7] [PMID] [PMCID]

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عفونت مایکوپلاسمایی در ریه گاو: شناسایی *مایکوپلاسما دیسپار* برای اولین بار در ایران

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زمینه مطالعه: اعضای جنس *مایکوپلاسما* به‌عنوان عوامل بیماری‌زای ایجادکننده بیماری تنفسی گاوها در دنیا شناخته می‌شوند.

هدف: هدف از مطالعهٔ حاضر تحقیق دربارهٔ عفونت مایکوپلاسمایی در بافت ریهٔ گاوهای کشتارشده در کشتارگاه صنعتی همدان با استفاده از روش‌های ملکولی و هیستوپاتولوژی بود.

روش کار: تعداد ۱۰۸ نمونهٔ بافتی از بخش قدامی- شکمی ریهٔ گاوها در فصول سرد و گرم جمع‌آوری‌شده و با روش‌های PCR و هیستوپاتولوژی آزمایش شدند. نمونه‌هایی که در آزمایش PCR مثبت بودند متعاقبا توسط آزمون Nested PCR برای یافتن گونه‌های *مایکوپلاسما بوویس* و *مایکوپلاسما دیسپار* بررسی شدند.

نتایج: از مجموع ۱۰۸ نمونه، تعداد ۹ (۸/۳۳٪) مورد حاوی DNA جنس *مایکوپلاسما* بود که در این میان، تعداد ۵ و یک مورد به ترتیب نشانگر توالی DNA خاص *مایکوپلاسما بوویس* و *مایکوپلاسما دیسپار* بودند. تغییرات پاتولوژیک نظیر ضایعات کازئونکروتیک، پنومونی بینابینی، برونکوپنومونی لوبی، و اتلکتازی برونشی در ۲۴ (۲۲/۲۲) نمونه مشاهده شد. تمامی ریه‌های با نتیجه PCR مثبت حداقل یک تظاهر پاتولوژیک را نشان دادند، لیکن همه بافت‌های دارای نشانه‌های پاتولوژیک دارای DNA گونه‌های *مایکوپلاسما* نبودند

نتیجه گیری نهایی: بر اساس نتایج این مطالعه می‌توان نتیجه‌گیری کرد که حضور *مایکوپلاسما بوویس* و در سطح پایین‌تر *مایکوپلاسما دیسپار* در جمعیت‌های گاو در منطقه غرب ایران نسبتا معمول بوده و لذا شایسته است ارتباط وجود این پاتوژن‌ها با مشکلات تنفسی موجود در گاوها مد نظر قرار گیرد.

واژه‌های کلیدی: بیماری تنفسی، *مایکوپلاسما*، *مایکوپلاسما بوویس*، *مایکوپلاسما دیسپار*، PCR آشیانه‌ای

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