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A molecular survey for detection of *Mycoplasma bovis* in bovine bulk milk samples of dairy farms in Hamedan, Iran

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Abstract

Mastitis is a well-recognized and costly disease of dairy cattle. *Mycoplasma bovis* is one of the most important causal agents of mastitis in dairy cows. In respect to the importance of mastitis caused by *Mycoplasma*, the objective of the present study was to evaluate of presence of *M. bovis* in bulk milk of dairy cattle farms of Hamedan province, Iran. For this purpose, a total of 125 bovine bulk tank milk samples were collected from 31 dairy farms of Hamedan, Iran. After that, California Mastitis Test (CMT) and Somatic Cell Count (SCC) were done on the milk samples. Then using DNA extraction kit, the total DNA was extracted from each sample. The PCR followed by nested PCR (nPCR) was performed for specific detection of *M. bovis.* Based on PCR for genus detection, totally, 19 out of 125 (15.2%) bulk tank milk samples were contaminated with *Mycoplasma* spp. In addition, 11 samples (8.8 %) were contaminated by *M. bovis* based on nested PCR results. Moreover, the results showed that 26 of 125 bulk milk samples (20.8 %) and 102 of 125 bulk milk samples (81.6 %) have high rate score by CMT and SCC, respectively. Statistical analysis showed a positive correlation between CMT and SCC results. In the present study, the presence of *M. bovis* in the bulk tank milk samples suggests that more hygiene practices are required to avoid transmitting this pathogen among dairy cow herds of Hamadan region. According to the presence of *Mycoplasma* in bulk tanks of this region, the present study suggested that the frequency of *Mycoplasma* contamination in individual cows be determined.

Key words: Mastitis, *Mycoplasma*, Bulk tank milk, Cow, Hamedan

Introduction

Bovine mastitis as an important disease in dairy cows is responsible for economic losses throughout the world (Halasa et al, 2007; Joshi et al, 2006). Mastitis is an inflammation of the mammary gland due to very vast factors including physical factors such as trauma and infectious factors such as various pathogenic bacteria. Several

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types of bacteria in both gram positive and gram negative can invade the udder, multiply and produce toxins that are harmful to the mammary gland (Bitew et al, 2010; Bradley 2002). Mastitis is responsible for major economic losses to dairy producers, resulting in reduced milk production, alteration in milk composition, discarded milk, increased replacement costs, treatment costs, and veterinary services (Halasa et al. 2007; Joshi et al. 2006).

More than 137 microbial species, subspecies and serovars have been isolated from the bovine mammary gland. However, most infectious bovine mastitis are caused by various species of streptococci, staphylococci, and Gram-negative rods, especially lactose-fermenting organisms and Mycoplasma spp. (Acharya et al, 2022).

Two different criteria may be used for classification of bovine mastitis: clinical symptoms (clinical or subclinical) and the mode of transmission (contagious or environmental) (Abrahmsén et al, 2014). Clinical mastitis is an inflammatory response to infection causing visibly abnormal milk (color, fibrin clots). Then, following the extension of the inflammation, changes in the udder (swelling, heat, pain and redness) may also be apparent. Finally, the agent cause of this type of mastitis may be systemic in all of the body. But in subclinical mastitis, infection is presence without apparent signs of local inflammation or systemic involvement (Mdegela et al, 2009).

Mycoplasmas are normal inhabitants of the upper respiratory airways, urinary and genital tracts, and the digestive system; however, they can become infectious and cause major diseases in dairy herds including mastitis and metritis (Torres et al, 2022). In dairy cattle, *M. bovis* is the most commonly isolated Mycoplasma species from bovine mastitis. This bacterium is a cause of significant economic loss in the dairy industry (Liu et al, 2020).

Mycoplasma spp. may spread from cow to cow through aerosol transmission and invade the udder subsequent to bacteremia. Contagious pathogens are spread during milking by milkers' hands or the liners of the milking unit (Barkema et al, 2009). The infected cows may shed billions of organisms into raw milk during clinical outbreaks. At other times, they may shed very few. For this reason, regular bulk tank milk screening (bacterial culture and molecular techniques) is suggested as a good tool to monitor a herd's Mycoplasma mastitis status. A positive result indicates that at least one cow has Mycoplasma mastitis (Gonzales et al, 2003). On the other hand, culture method has disadvantages: Preparation of the medium for Mycoplasma culture is a tedious and time-consuming process and subsequent subculturing for identification may take 5–10 days (Ghadersohi et al, 1997). Based on the previous studies, polymerase chain reaction (PCR) was accepted as a quick and reliable method for accurate diagnostic method for detecting Mycoplasma strains in milk samples (Baird et al, 1999).

Accordingly, the aim of the present study was to determine the presence of Mycoplasma spp. and subsequently *M. bovis* in bulk milk samples of dairy farms in Hamedan province, Iran. The detection process was carried out by PCR for genus- and species-specific sequences. Bulk milk samples were examined by CMT and somatic SCC for measurement of correlation rate between these mastitis-related test and presence of Mycoplasma.

Materials and Methods

Bulk Tank Milk Samples

In the period of March 2017 to February 2019, a total of 125 bulk tank milk samples were collected from 31 dairy farms of Hamedan province, Iran. In this study, the dairy farm was enrolled by more than 50 dairy cows. The sample collection was done in all four seasons. Milk sample (30 ml) was collected from the thoroughly mixed bulk

tank and transported to sterile tubes under aseptic condition. The sample was put on the ice container box and transported to laboratory as soon as possible and was processed for CMT and SCC tests. Two × One ml of each milk sample were aliquoted and transferred into sterile vial and frozen until DNA isolation.

California Mastitis Test

The California Mastitis Test (also known as the California Milk Test) works by using a reagent which disrupts the cell membrane of somatic cells present in the milk sample. After disruption of the cell membrane, the DNA in those cells is reacting with the test reagent. It is a simple but very useful technique for detecting subclinical mastitis. For CMT test, about 2 ml milk from each sample was squirted into well of the paddle; Then added an equal amount of CMT solution (Shirazma, Iran) was added to well of the paddle. The paddle rotated in a circular motion to thoroughly mix the contents for 10 seconds. Based on the viscosity of paddle content, the reaction is scored a scale of 0 (the mixture remaining unchanged and can easily be shaken) to 3 (an almost-solid gel forming), with a score of 2 or 3 being considered a positive result (Leach et al, 2008).

Somatic Cell Count

The Somatic Cell Count is a main indicator of milk quality. The majority of somatic cells are leukocytes and small number of epithelial cells. The presence of these cells is increasing in milk usually as an immune response to an infectious mastitis. For SCC, Direct Microscopic Somatic Cell Count (DMSCC) was performed (Nagy et al, 2013). In brief, smears (10 µL) in duplicate were prepared on somatic cell slides, air-dried for 15 min, and treated with Xylol for 3 min. Then, the slides were immersed in absolute ethanol for 5 min. Smears were stained by adding 2 drops of Giemsa stain (Sigma-Aldrich, United States) for 1 min. Then, the stain was removed with absolute ethanol (Pars Alcohol, Iran) for 5-10 sec. The slides were left in laboratory temperature for 20 min to dry; and somatic cells were counted on 10 fields of one smear using a light microscope at 1000× magnification. Final values (cells/mL) were calculated using the following equation: SCC/mL = (Σ10 fields) × 500000. The values up to 3×10^5 were considered as high SCC.

DNA Extraction

A total DNA was extracted from milk directly. A 1000 µL of each milk sample was added to sterile tube and centrifuging at 13000 × g for 60 min. The supernatant was decanted by adding a 1000 µl DNase-Free distilled water to the pellet and re-suspend the cells were resuspended completely by vortexing or pipetting. The tubes were centrifuged at 13000 × g for 60 min and supernatant was removed. DNA extraction was performed on the harvested cells by DNA extraction kit (Yektatajhiz, Tehran, Iran) according to the manufacturer's recommendations.

PCR and nested PCR for detection of *Mycoplasma bovis*

In the first step, the conventional PCR was done for detection of Mycoplasma genus. The detection of *M. bovis* was followed by nPCR in the next step. A genus-specific Mycoplasma sequence (general prokaryotic oligonucleotide (GPO) was used for amplification according to the primers described previously (Table 1). The PCR mixture for conventional PCR (25 ml) contained 1 mM forward and reverse primers, dNTP mix (100 mM each of dATP, dCTP, dGTP and dTTP), 1 U of Taq DNA polymerase, 2.5 ml PCR buffer (10X), 5 ml DNA template and 14 ml sterile distilled water. Thermal conditions for the PCR were described in Table 2. PCR products (8 ml) were analyzed on 1.5% (wt/v) agarose gel contain ethidium bromide (0.5 $mg.ml^{-1}$), and visualized under ultraviolet transillumination and photographed using Gel Doc apparatus. A negative control

(water) and a positive control (*Mycoplasma synoviae*) were included in each PCR test.

Nested PCR was performed for detection of bovine species by species specific primer (PpSM5) (Table 1) as described by Hotzel et al. (1999). The PCR product was diluted with sterile distilled water (1:10 v/v). The diluted DNA was used as template for nPCR. The nested PCR components were similar to those mentioned above. Thermal cycles of nPCR are shown in Table 2. Finally, as no control strain was available for *M. bovis* species, the nested PCR product was confirmed by DNA sequencing.

Statistical analysis

Statistical analysis was made with by SPSS software version 17.00. The correlation between CMT and SCC was calculated by Spearman's rank correlation test. The mean difference was considered significant at $P < 0.05$.

Table 1. The sequences of primers used in the study

Genes' name	Primer	PCR product (bp)	Reference
GPO	-TTTTAGCTCTTTTTGAACAAAT-3	1013	(Van Kuppeveld *et al.* 1992)
	55-GGCTCTCATTAAGAATGTC-3		
PpSM5	5-CCAGCTCACCCTTATACATGAGCGC-3	442	(Hotzel *et al.* 1999)
	5- TGACTCACCATTTAGACCGACTATTTCAC-3		

Table 2. The thermal conditions of PCR were used in the study

Genes' name	Initial denaturation	Repeat cycles (35 cycles)	Final extension
GPO	94 °C for 5 min	94 °C for 1 min 54 °C for 1 min 72 °C for 1 min	72 °C for 10 min
PpSM5	94 °C for 11.5 min	94 °C for 1 min 54 °C for 1 min 72 °C for 2 min	72 °C for 5 min

Results

In the present study, a total of 125 milk samples were collected from 31 dairy herds during the hot-dry season (mid-spring, mid-summer) and cold-wet season (mid-autumn and mid-winter). The results of PCR indicated that out of the 125 bulk milk samples, 19 (15.2%) were contaminated with Mycoplasma spp. Moreover, the nested PCR test revealed that out of 19 samples, 11 (57.2%) were *M. bovis* species (Figure 1).

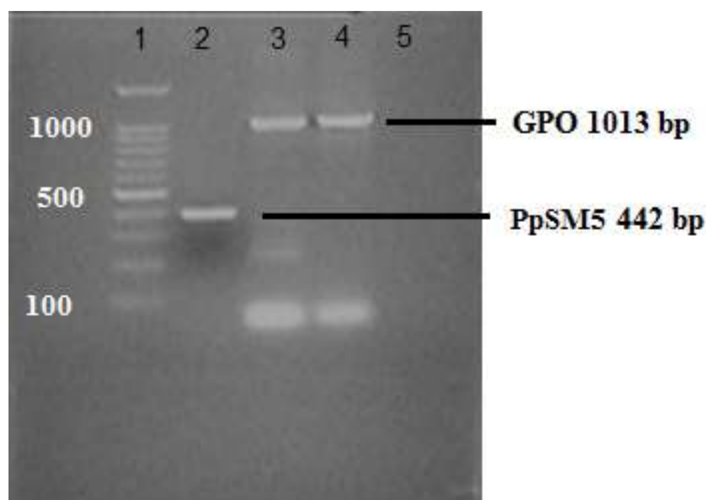

Figure 1: PCR and Nested PCR amplification for *M. bovis* detection. Lane 1: 100 bp DNA ladder, Lane 2: positive sample for *M. bovis*, Lanes 3 and 4: positive samples for Mycoplasma, Lane 5: negative control (no template DNA).

Based on the National Center for Biotechnology Information (NCBI), DNA sequences of PCR products were found to have 99% identity, corresponding to GenBank sequences (CP042939.1) of *M. bovis* (Figure 2).

Mycoplasmopsis bovis strain NADC59 chromosome, complete genome

Sequence ID: CP042939.1 Length: **1183547** Number of Matches: **1**

Range 1: 133715 to 134106 GenBank Graphics ▼ Next Match ▲ Previous M

Score	Expect	Identities	Gaps	Strand
712 bits(385)	0.0	390/392(99%)	1/392(0%)	Plus/Plus

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Query  1       ATACCTG-AAATGATGATGAGAGATTATTCTCAATTCAAGGAACCCCACCAGATATGGCA  59
               ||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  133715  ATACCTGAAAATGATGATGAGAGATTATTCTCAATTCAAGGAACCCCACCAGATATGGCA  133774

Query  60      AACTTACCTATCGGTGACCCTTTTGCACCTAGAAATGACTTTGCCTTAGAAATTGACTAT  119
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  133775  AACTTACCTATCGGTGACCCTTTTGCACCTAGAAATGACTTTGCCTTAGAAATTGACTAT  133834

Query  120     GAAAAAGAACCACCATTAATTGAAATTAATAGTCATCATAAAGCAGCAACGTGACTACTT  179
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  133835  GAAAAAGAACCACCATTAATTGAAATTAATAGTCATCATAAAGCAGCAACGTGACTACTT  133894

Query  180     CACCCTGATGCACCAAAAATACAAGACCAAAAGAATTAGAACATAGACCAAAAAGTTTT  239
               |||||||||||||||||||||||||||||||||||||||||||||||| |||||||||||
Sbjct  133895  CACCCTGATGCACCAAAAATACAAGACCAAAAGAATTAGAACATAGACTAAAAAGTTTT  133954

Query  240     AGAAAGGTATTTAAAGACGATGAAGAATAACGACAATAAAAAAGTCATTTTAGAAATTCA  299
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  133955  AGAAAGGTATTTAAAGACGATGAAGAATAACGACAATAAAAAAGTCATTTTAGAAATTCA  134014

Query  300     AGATCTTAAAAAGTACTTTTTAAATAACGGTAAGGTCAACAAAGCTGTTGATGGTGTGTC  359
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  134015  AGATCTTAAAAAGTACTTTTTAAATAACGGTAAGGTCAACAAAGCTGTTGATGGTGTGTC  134074

Query  360     ATTTAAATTACATGAAGGTGAAATAGTCGGTC  391
               ||||||||||||||||||||||||||||||||
Sbjct  134075  ATTTAAATTACATGAAGGTGAAATAGTCGGTC  134106
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Figure 2. Sequence of PCR product and its blast result based on NCBI database

The number of positive sample related to the Mycoplasma spp. was 2, 2, 6 and 9 in spring, summer, autumn and winter, respectively. In related to *M. bovis* the number of positive sample was 1, 2, 1 and 7 in spring, summer, autumn and winter, respectively.

The CMT and SCC tests were performed on the samples. The results showed that 102 out of 125 (81.6 %) milk samples have high rate score in SCC. Moreover, 26 out of 125 (20.8 %) milk samples showed positive results by CMT. The details of the results are presented in table 3. A positive correlation was found between CMT and SCC (r: 94.79, P < 0.05).

Table 3. CMT and SCC with high score samples during the seasons

Seasons	Milk samples	High CMT score (%)	High SCC score (%)
Spring	31	10 (32.25)	23 (74.19)
Summer	31	4 (12.90)	25 (80.64)
Autumn	31	5 (16.12)	24 (77.41)
Winter	32	7 (21.87)	30 (93.75)
Total	125	26 (20.8)	102 (81.6)

It should be noted that the most Mycoplasma contamination between four seasons is related to winter, which has the most SCC rate score. As a matter of fact, no statistically significant difference was observed between the number of samples contaminated by Mycoplasma spp. DNA and CMT results.

Discussion

In lactating cows, *Mycoplasma* mastitis leads to decreased milk production and poor milk quality (high somatic cell count) (Torres et al, 2022). In bovine mastitis caused by Mycoplasma, the etiological agent can spread from cow to cow during milking or by treating with contaminated therapeutic agents. Several species of Mycoplasma can infect cattle, but *M. bovis* is the most frequent cause of Mycoplasma mastitis (Itoh et al, 2023).

Mastitis, due to Mycoplasma, may be clinical or subclinical, acute or chronic forms. In cows with clinical mastitis, systemic signs of disease (fever and off-feed) are rare. In bovine mastitis, Mycoplasma may be shed in high numbers in milk; for this reason, screening of bovine milk for Mycoplasma bacteria or its genome is valuable (González et al. 2003). Individual cow milk, bulk tank samples, or sequential sampling after milking groups can be tested for detection of this pathogen. The molecular technique such as PCR can be used on bulk tank milk samples. This test detects minute quantities of specific Mycoplasma DNA, has good sensitivity and specificity and also requires much less time to complete (Tretter, 2016).

This is the first study done on bulk tank milk of Hamadan province in central west of Iran, in order to establish the presence of *M. bovis* and its relation with SCC and CMT values. Our present findings showed high value of CMT in milk samples, indicating the presence of subclinical mastitis in dairy farm of this region. Although the CMT does not identify the type of bacterial causing agent of mastitis, it is useful to identify subclinical mastitis (Barkema et al, 1999). Based on the bacteriological culture of milk samples as the gold standard test, the sensitivity and specificity of CMT for detection of infectious mastitis are reported 82.4% and 80.6%, respectively (Dingwell et al, 2003).

Since the presence of various somatic cells in milk is principle of CMT test, the result of this test can be related to SCC value. The relationship (between CMT and SCC) was 94.79% in the present study. Optimal sensitivity and specificity of SCC as the indicator of presence of

intramammary infection (IMI) were estimated at 73% and 86%, respectively (Dohoo *et al.* 1991). Similar to CMT, the sensitivity and specificity of SCC are not very high for detection of individual cow mastitis (Dingwell *et al.* 2003); but the results may give valuable data about prevalence of subclinical mastitis in dairy farms when SCC is done in corporation with CMT as screening tests (Sargeant et al, 2001).

Identification of Mycoplasma bacteria, based on culturing on selective media, is difficult; because these organisms are sensitive to drying, pH changes, and extended refrigeration or freezing periods and has specific condition for culture. PCR testing by their sensitivity, specificity, cost, and speed of results, is an attractive method for detection of this Mycoplasma spp. in milk samples from bulk tanks (Tretter, 2016).

The results of our study showed 15.2% of milk samples were contaminated with Mycoplasma spp. out of which 11 of 19 positive samples (57.2%) were *M. bovis*. The results revealed that farms of Hamedan region are contaminated with Mycoplasma and management control related to this bacterium should be performed.

In Ghazaei's (2006) study, using modified Hayflick method, *M. bovis* was isolated from 48.75% of the clinical mastitis samples and 60% of the bulk-tank milk samples in Moghan region of Ardabil, Iran. In a similar study performed by Dabiri et al. (2017), using nested PCR, the presence of *M. bovis* in bulk tank milk samples was not reported in Mashhad, Iran. Besides, the researchers reported that the investigated samples were contaminated with *M. canadense* and *M. yeatsii*.

We demonstrated that all samples being positive for Mycoplasma in PCR, have high SCC. In addition, positive correlations were observed between Mycoplasma presence and SCC values. Fox et al. (2003) declared that milk quality components were not strongly related to the presence of Mycoplasma in bulk tank milk. Gonzalez et al. (1992) reported that 52±65% of cows were culture positive for *Streptococcus agalactiae* and *Staphylococcus aureus* on a herd with a high bulk milk SCC but the animals were refractory to intramammary therapy. After several weeks, most of the cows were found to be culture positive for *M. bovis*. Ghadersohi et al. (1998) investigated the association between detection of *M. bovis* and SCC in composite milk from bulk milk samples and reported that there was 95% association between presence of *M. bovis* and SCC. They noted that *M. bovis* infection can increase the mastitis infection rates caused by other pathogens and environmental microorganism (Ghadersohi et al. 1999). It seems that the presence of Mycoplasma in bovine udder and its role as a predisposing factor for other bacteria may influence the composition of milk and increase the somatic cells and therefore increase SCC and CMT. According to the presence of *M. bovis* in bulk tank milk samples of Hamedan province, more hygiene practices can be suggested.

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Conflict of Interest

The authors declare no conflict of interest.

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References

Abrahmsén, M., Persson, Y., Kanyima, B. M. and Båge, R. (2014). Prevalence of subclinical mastitis in dairy farms in urban and peri-urban areas of Kampala, Uganda. *Tropical Animal Health and Production*, 46: 99-105.

Acharya, D., Parida, P., Mohapatra, H.S., Sahoo, S.L. and Rout, J.R.. (2022). Bovine Mastitis: Causes and Phytoremedies. *Journal of Pure Applied Microbiology*, *16*(4),2259-2269.

Baird, S. C., Carman, J., Dinsmore, R. P., Walker, R. L. and Collins, J. K. (1999). Detection and identification of Mycoplasma from bovine mastitis infections using a nested polymerase chain reaction. *Journal of Veterinary Diagnostic Investigation,* 11: 432-435.

Barkema, H., Deluyker, H., Schukken, Y. and Lam, T. (1999). Quarter-milk somatic cell count at calving and at the first six milkings after calving. *Preventive Veterinary Medicine*, 38: 1-9.

Barkema, H., Green, M., Bradley, A. and Zadoks, R. (2009). Invited review: The role of contagious disease in udder health. *Journal of Dairy Science,* 92: 4717-4729.

Bitew, M., Tafere, A. and Tolosa, T. Study on bovine mastitis in dairy farms of Bahir Dar and its environs. (2010). *Journal of Animal and Veterinary Advances,* 9: 2912-2917.

Bradley, A. J. (2002). Bovine mastitis: an evolving disease. *The Veterinary Journal,* 164: 116-128.

Dabiri, M., Mirshokraei, P., Rad, M., Khoramian, B. (2017). Detection of Mycoplasma bovis in bulk tank milk samples by nested PCR in Mashhad, Iran. *Iranian Journal of Veterinary Science and Technology*, 9(2). 28-32.

Dingwell, R. T., Leslie, K. E., Schukken, Y. H., Sargeant, J. M. and Timms, L. L. (2003). Evaluation of the California mastitis test to detect an intramammary infection with a major pathogen in early lactation dairy cows. - *Canadian Veterinary Journal*, 44: 413-415.

Dohoo, I. R. and Leslie, K. (1991). Evaluation of changes in somatic cell counts as indicators of new intramammary infections. *Preventive Veterinary Medicine*, 10: 225-237.

Fox, L., Hancock, D., Mickelson, A., Britten, A. and Kaaden, O. R. (2003). Bulk tank milk analysis: factors associated with appearance of Mycoplasma sp. in milk. *Journal of Veterinary Medicine, Series B,* 50: 235-240.

Ghadersohi, A., Coelen, R. and Hirst, R. (1997). Development of a specific DNA probe and PCR for the detection of *Mycoplasma bovis*. *Veterinary Microbiology* 56: 87-98.

Ghadersohi, A., Hirst, R., Forbes-Faulkener, J. and Coelen, R. (1999). Preliminary studies on the prevalence of *Mycoplasma bovis* mastitis in dairy cattle in Australia. *Veterinary Microbiology,* 65: 185-194.

Ghazaei, C .(2006). Mycoplasmal mastitis in dairy cows in the Moghan region of Ardabil State, Iran: short communication. *Journal of the South African Veterinary Association* ,77: 222-223.

Gonzales, R. and Wilson, D. J. (2003). Mycoplasmal mastitis in dairy herds. *NMC Newsletter*, 26: 4-4.

Gonzalez, R. N., Sears, P. M., Merrill, R. A. and Hayes, G. L (1992). Mastitis due to Mycoplasma in the state of New York during the period 1972-1990. *The Cornell Veterinarian*, 82: 29-40.

González, R. N. and Wilson, D. J. (2003). Mycoplasmal mastitis in dairy herds. *Veterinary Clinics of North America: Food Animal Practice,* 19: 199-221.

Halasa, T., Huijps, K., Østerås, O. and Hogeveen, H. (2007). Economic effects of bovine mastitis and mastitis management: A review. *Veterinary Quarterly,* 29: 18-31.

Hotzel, H., Heller, M. and Sachse, K. (1999). Enhancement of *Mycoplasma bovis* detection in milk samples by antigen capture prior to PCR. *Molecular and Cellular Probes,* 13: 175-178.

Itoh, M., Hata, E., Iguchi, H., Takahashi, E., Hassan, H. and Yamada, K., 2023. Dry Period Therapy Based on Antimicrobial Susceptibility for Cows with Subclinical Mastitis Caused by *Mycoplasma bovis*. *Journal of Current Veterinary Research*, *5*(1).62-68.

Joshi, S. and Gokhale, S. (2006). Status of mastitis as an emerging disease in improved and periurban dairy farms in India. *Annals of the New York Academy of Sciences* 1081: 74-83.

Leach, K., Green, M., Breen, J., *et al.* (2008). Use of domestic detergents in the California mastitis test for high somatic cell counts in milk. *Veterinary Record: Journal of the British Veterinary Association*. 163.

Liu, Y., Xu, S., Li, M., Zhou, M., Huo, W., Gao, J., Liu, G., Kastelic, J.P. and Han, B. (2020). Molecular characteristics and antibiotic susceptibility profiles of Mycoplasma bovis associated with mastitis on dairy farms in China. *Preventive Veterinary Medicine*, *182*, 105106.

Mdegela, R., Ryoba, R., Karimuribo, E., *et al.* (2009). Prevalence of clinical and subclinical mastitis and quality of milk on smallholder dairy farms in Tanzania. *Journal of the South African Veterinary Association* 80: 163-168.

Nagy, P., Faye, B., Marko, O., Thomas, S., Wernery, U. and Juhasz, J. (2013). Microbiological quality and somatic cell count in bulk milk of dromedary camels (Camelus dromedarius): Descriptive statistics, correlations, and factors of variation. *Journal of Dairy Science,* 96: 5625-5640.

Sargeant, J., Leslie, K., Shirley, J., Pulkrabek, B. and Lim, G. Sensitivity and specificity of somatic cell count and California Mastitis Test for identifying intramammary infection in early lactation. -*Journal of dairy science* 84: 2018-2024.

Torres, F.D., Madureira, K.M., da Silva, K.N., Azevedo, C., de Moura, T.M., Skorei, M.R. and Gomes, V. (2022). Influence of Mycoplasma bovis infection on milk production and quality of Holstein dairy cows. *Journal of Dairy Research*, *89*(4),416-418.

Tretter, E (2016). Comparison of PCR and culture methods for detecting mastitis causing mycoplasma in bulk tank milk from commercial dairy herds. *Joint Annual Meeting*. Asas 2016.

Van Kuppeveld, F., Van der Logt, J., Angulo, A., *et al.* (1992). Genus-and species-specific identification of mycoplasmas by 16S rRNA amplification. *Applied and Environmental Microbiology* 58: 2606-2615.

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تشخیص مولکولی *مایکوپلاسما بویس* در نمونه‌های تانک شیر در گاوداری‌های همدان، ایران

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چکیده

بیماری ورم پستان یک بیماری شناخته شده و پرهزینه گاوهای شیری است و باکتری *مایکوپلاسما بویس* یکی از مهم‌ترین عوامل ایجاد ورم پستان در گاوهای شیری می‌باشد. با توجه به اهمیت *مایکوپلاسما* در ایجاد ورم پستان، هدف از انجام مطالعه حاضر، بررسی مولکولی حضور *مایکوپلاسما بویس* در تانک شیر در گاوداری‌های شیری استان همدان بود. در مجموع ۱۲۵ نمونه شیر از تانک‌های مخزن شیر از ۳۱ گاوداری اطراف همدان جمع‌آوری شد. تست ورم پستان کالیفرنیا (CMT) و شمارش سلول‌های سوماتیک (SCC) بر روی نمونه‌های شیر انجام شد. سپس با استفاده از کیت استخراج، DNA کل از نمونه‌های شیر استخراج شد. آزمون PCR و به دنبال آن Nested PCR (nPCR) برای تشخیص اختصاصی *مایکوپلاسما بوویس* انجام شد. در مجموع بر اساس PCR اختصاصی جنس، ۱۹ مورد از ۱۲۵ نمونه شیر (۱۵/۲ درصد) به گونه‌های مختلف *مایکوپلاسما* آلوده بودند. در حالی که بر اساس نتایج nPCR، ۱۱ مورد (۸/۸ درصد) به عنوان *مایکوپلاسما بویس* تشخیص داده شد. نتایج نشان داد که ۲۶ مورد از ۱۲۵ نمونه شیر (۲۰/۸ درصد) و ۱۰۲ مورد از ۱۲۵ نمونه شیر (۸۱/۶ درصد) به ترتیب دارای امتیاز بالای CMT و SCC بودند. تجزیه و تحلیل آماری، همبستگی مثبت بین CMT و SCC را نشان داد. در مطالعه حاضر، حضور *مایکوپلاسما بویس* در نمونه‌های شیر فله‌ای، حاکی از آن است که برای جلوگیری از انتقال *مایکوپلاسما بویس* بین گله‌های گاو شیری منطقه همدان، رعایت موارد بهداشتی بیش‌تر مورد نیاز است. با توجه به حضور باکتری *مایکوپلاسما* در تانک شیر، پیشنهاد می‌شود که آلودگی به این باکتری در گاوهای این منطقه بررسی شود.

کلمات کلیدی: ورم پستان، *مایکوپلاسما*، شیر تانک مخزن، گاو، همدان

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