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Molecular detection of *Mycoplasma* spp. from camel's milk

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Abstract

The dromedary camels are essential because of their multiple uses for transport, wool, meat, and milk production. Camel's milk is considered exceptional nutrition, so it uses for therapeutic and high nutrition. Mastitis may affect camel's milk production, cause significant economic loss, and is associated with zoonotic disease. The study aimed to detect the prevalence of *Mycoplasma spp.* especially *M. bovis* in camel's milk using the polymerase chain reaction (PCR) method as a primary molecular diagnostic technique. Fifty milk samples were collected from she camels suffering from subclinical mastitis in Iraq. The result of the current study declared that 26.66% of the camel's milk samples were positive for *Mycoplasma*. In contrast, the prevalence rate of *Mycoplasma* (*M.*) *bovis* in all the samples was 61.53%. The study concludes that *Mycoplasma* and especially *M. bovis* are considered one of the bacteria that cause subclinical mastitis in camels, and using the PCR method is regarded as a more rapid, simple, and current for detecting the Mycoplasma bacteria.

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Introduction

The dromedary camel, or Arabian one-humped camel, is scientifically known as *Camelus dromedarius* and is found in many countries in Asia and Africa, especially those with desert and semidesert climate (1-4). Camels are essential because they are used as a means of transport in the desert and consider one source of economic income that comes from wool, milk, and meat production (1,5,6) and from using camels in racing (7,8). Camel's milk considers an exceptional nutritional food because of its high nutritional and therapeutic uses (9-13). Although camels consider more resistant to many diseases that occur in other animals (14), many diseases can affect camels and lead to crucial economic loss (15). In many countries, including Iraq, camel's milk production was affected by mammary gland infection, also known as mastitis (16). Clinical mastitis may be associated with the change in milk colour and composition associated with swelling, painful mammary glands, and/or remaining without any apparent signs of subclinical mastitis (16-20). Many etiological agents can cause camels mastitis, including *Escherichia coli, Staphylococcus spp, Streptococcus agalactiae, Micrococcus spp, Arcanobacterium spp, Mannhiemia spp. Salmonella spp, Klebsiella spp.* (21), and finally, *Mycoplasmas spp*. (15,22). *Mycoplasma spp.* was a cell wall deficient prokaryotes bacteria classified under the class Mollicutes, and its definitive as the smallest organism capable of self-replicating (14). Most of the *Mycoplasma* infections in camels remain undiagnosed, partly because of the scarcity of camel's *Mycoplasma* studies (23) and because the *Mycoplasma* classical diagnosis method is complex and takes a long time. Moreover, the *Mycoplasma* serological tests could give false results because of the cross-reactivity of *Mycoplasma spp.* with other closely related bacterial species (14, 24,25). In recent decades, molecular methods have been used to diagnose *Mycoplasma* infection. The introduction of molecular diagnoses using the polymerase chain reaction (PCR) method with a specific primer provides a rapid and accurate tool to detect and identify *Mycoplasma* infections (14,15,26-29). The previous studies reported that

many species of *Mycoplasma* were associated with mammary gland infection in cows, including *M. Bovis, M. californium, M. bovigenitalium, M. alkalescens, and M. canadense* (30). *M. bovis* is the most widely contagious species that spreads rapidly throughout the herd (31) that causing eye, joint, respiratory, or urogenital infection (32) and is mainly associated with *M. bovis* mastitis (18).

Although camels are an essential source of zoonotic diseases such as the Middle East respiratory syndrome and other bacterial diseases like Mycoplasmosis (7,33), very few articles focus on *Mycoplasma* mastitis, especially that caused by *M. bovis* in camels in Iraq and the Arabic area. The current study aimed to detect Mycoplasma's prevalence, especially *M. bovis* in camel's milk.

Materials and methods

Ethical approval and Data collection permit

All milk sample was collected after taking approval from the she-camel owners for sampling, all animals were treated ethically during the milk sampling, and according to the authorized ID. UM. Vet. 2022.013 which was provided by the Mosul University/college of veterinary medicine/Institutional Animal Care and Use Committee.

Sampling

Fifty milk samples were collected from one-humped camels distributed in the Badia Al Jazeera area in Al- Anbar and Nineveh province from February to June 2022 (Figure 1). The samples were collected from She-Camels that were suffering from subclinical mastitis. A 20 ml of each milk sample was collected in a sterile test tube and transported under the cooling condition (in a cool box containing Co_2 ice) to the Department of Microbiology and Department of Veterinary Public Health, College of Veterinary Medicine, Mosul University. All milk samples were saved in deep freeze until used for DNA extraction.

Mycoplasma DNA extraction

The DNA of the genus *Mycoplasma*. was extracted from milk samples by using the gSYNC™ Geneaid extraction kit (Geneaid Biotech Ltd, Taiwan) according to company instructions. Two primer pairs were used: a universal primer set to detect the genus *Mycoplasma* and a second specific-species primer for detecting *M. bovis*. All the primers were synthesized and supplied by Bioneer cooperation / Korea, according to the sequence noted by (34,35), to produce amplicon sizes of 285 bp and 232 bp, respectively (Table 1).

Figure 1: Geographical distribution map of camel milk sample.

Table 1: Primers used to detect genus *Mycoplasma* and *M. bovis*

Primer	Sequence (-) Product	Amplification product size	References
Myco. spp. F	5-GGGAGCAAACACGATAGATACCCT-3	285 bp	34
Myco. spp. R	5-TGCACCATCTGTCACTCTGTTACCCTC-3		
M. bovis F	5-ATATTGAAAAAGTTATAT-3	232 bp	35
M. bovis R	5-TAAACTCTCAGAATCTA-3		

The DNA amplification was done by using a 25 µl mixture containing (5.5 µl dd distal water, 12.5 µl 2.5X PCR master mix, 1 µl from both forward and reverse primer, 5 µl extracted *mycoplasma* DNA, 1.5 µl $MgCl_2$); the amplification programs was set as describer (Table 2). All the PCR products were migrated in 1.5% agarose (Biometra/Germany), and DNA bands were visualized by a UV transilluminator (Biometra /Germany) and photographed.

Table 2: The amplification programs used to detect the genus *Mycoplasma* and *M. bovis*

Stage	Temperature ℃	Time min.	Cycle No.
Initial denaturation	95	5	1
Denaturation	95	0.3	
Annealing	59*, 40 **	0.5	30
Extension	72	0.5	
Final extension	72	5	1

* Temperature used for amplification of Mycoplasma genus, ** Temperature used for amplification of the specific-species gene of *M. bovis*.

Results

Our study shows that 26.66% (13/50) of camel's milk samples were infected with *Mycoplasma spp*. In addition, the prevalence rate of *M. bovis* from milk samples was high and reached 61.53% (8/13) from all positive milk Mycoplasma infections, the result showed that total isolation for *M. bovis* from all milk samples 16 %(8/50).

The amplification of PCR product in 1.5% agarose gel reveals one high specific band in 258 and 232 bp for *Mycoplasma* and *M. bovis* respectively (Figures 2 and 3).

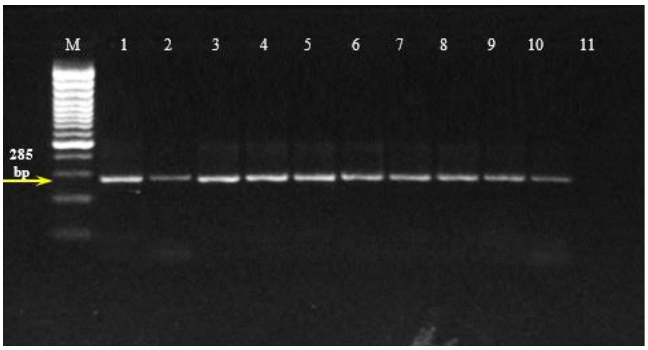

Figure 2: Amplified PCR products of *Mycoplasma* Gene (M: Molecular base ladder, 1: Positive control, 11: Negative control, 2-10: milk sample).

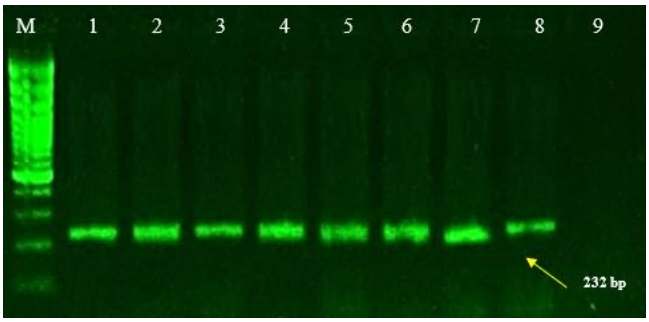

Figure 3: Amplified PCR products of *M. bovis* gene (M: Molecular base ladder, 1: Positive control, 9: Negative control, 2-8: milk sample).

Discussion

Camel's milk shows great economic importance nowadays because of the long lactating period of camels with the high nutrient values and therapeutical uses of its milk (9), so any disease in the mammary gland that affects milk production and causes significant loss is associated with zoonotic infection and cost of animal's welfare (36). Many microorganisms can grow and multiply in camel's milk, especially after entering the teat channel, which comes from either the teat/mammary gland surface, milker hand, or the environment that causes camel's mastitis (37-39). Bacterial camel's mastitis can be either clinical and/or subclinical. The subclinical mastitis was most crucial as it did not show any clinical signs and did not cause any noticeable change in the udder or milk, farther that subclinical mastitis comes before clinical mastitis and may take a long period to diagnose, which causes the animals' source for environmental contamination and spread of disease for other animals (40,41). Many studies focus on camel's mastitis and show that about 25% were subclinical cases in Ethiopia (12), 16.6% in Saudi Arabia (42), and 11.7% in the United Arab Emirates (43). Finally, 25.8% in Egypt (41) were associated with different bacterial isolates (17,37). This percentage was near to that recorded in our study, reaching about 26% from all milk samples, which indicates highly spread of *Mycoplasma* infection in milk.

In our country, very few articles were published dealing with camel's mastitis, and no research about the role of *Mycoplasma* bacteria in camel's mastitis was recorded. Our study firstly isolated *M. bovis* from camel's milk with a high percentage reaching 61.5% of the total milk sample. This indicates that *M. bovis* can be the primary source of subclinical camel's mastitis infection. *M. bovis* was globally spread and able to cause many diseases, including pneumonia, keratoconjunctivitis, abortion, infertility, and mastitis (32,44,45). Most mammary gland infection caused by *M. bovis* was subclinical and associated with a respiratory infection. Mahmoud *et al*. (46) reported that *M. bovis* was found in the respiratory tract in both diseased and healthy camels in Egypt (46). Iraqi articles focus on the isolation of other bacteria rather than *Mycoplasma spp.* from mastitis camel's milk which used conventional bacteriological methods which not suitable to diagnose Mycoplasma as it takes a long time to grow this many explain partially leaks of Iraqi articles dealing with camel's *Mycoplasma* mastitis. Unlike our result, Tigani *et al.* (47) was unable to diagnose *M. bovis* in camels' gangrenous mastitis by PCR in their study conducted in the United Arab Emirates (47). This may be due to some PCR inhibiter in a sample, like sampling time and /or contamination with other bacteria, which lead to false-negative results (35). Only 61.53% of Mycoplasma milks *M. bovis* caused the infection, indicating that other *Mycoplasma* species may cause subclinical mastitis in camels. This agrees with (22) how reported isolation of *M. arginine* from camel sub-clinical mastitis in Sudan.

Conclusion

This was the first local study in Iraq that detected the ability of *Mycoplasma*, especially *M. bovis* to cause subclinical camel mastitis by using the PCR method as the primary diagnostic molecular method.

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Conflict of interest

There was no conflict of interest.

References

1. Abdelazeem WM, Zolnikov TR, Mohammed ZR, Saad A, Osman KM. Virulence, antimicrobial resistance, and phylogenetic analysis of zoonotic walking pneumonia *Mycoplasma arginine* in the one-humped camel (*Camelus dromedarius*). Acta Tropica. 2020;207:105500. DOI: 10.1016/j.actatropica.2020.105500
2. Park YW, Haenlein GW, Wendroff WL. Handbook of milk of nonbovine mammals. USA: Blackwell Publishing; 2006. 297–344 p.
3. Abera T, Legesse Y, Mummed B, Urga B. Bacteriological quality of raw camel milk along the market value chain in Fafen zone, Ethiopian Somali regional state. BMC Res Notes. 2016;9(1):285-291. DOI: 10.1186/s13104-016-2088-1
4. Al-Fatlawi AA, Alfatlawi MA. Morphological and molecular identification of *Parabronema skrjabini* of camels (*Camelus dromedary*) in Najaf province. Iraq J Vet Sci. 2021;35(3):507-512. DOI: 10.33899/ijvs.2020.127101.1459
5. Sheet OH, Jwher DM, Al-Sanjary RA, Alajami AD. Direct detection of *Staphylococcus aureus* in camel milk in the Nineveh governorate by using the PCR technique. Iraq J Vet Sci. 2021;35(4):669-672. DOI: 10.33899/ijvs.2020.127725.1524
6. Kaaboub EA, Ouchene N, Ouchene NA, Dahmani A, Ouchtati I, Haif A, Khelef D. Investigation of the principal vectors of abortive diseases in one-humped camels (*Camelus dromedarius*). Iraq J Vet Sci. 2021;35(3):411-415. DOI: 10.33899/ijvs.2020.126914.1415
7. Mohammadpour R, Champour M, Tuteja F, Mostafavi E. Zoonotic implications of camel diseases in Iran. Vet Med Sci. 2020;6(3):359-381. DOI: 10.1002/vms3.239
8. Al-Shorepy SS. Identification of environmental factors affecting the racing performance of race camels in the United Arab Emirates. Emir J Food Agric.2011;23(5):424-430. [available at]
9. Al Salihi K, al Khatib MM, Alkoofee WM. Physicochemical properties of Iraqi dromedary camel's milk. Basra J Vet Res. 2017;16(2):45-53. DOI: 10.33762/bvetr.2017.143531
10. Geresu MA, Leliso SA, Liben GW. Camel mastitis: Prevalence, risk factors, and isolation of major bacterial pathogens in Gomole district of Borena zone, southern Ethiopia. Vet Med Int. 2021;2:1-11. DOI: 10.1155/2021/9993571
11. Abera M, Abdi O, Abunna F, Megersa B. Udder health problems and major bacterial causes of camel mastitis in Jijiga, eastern Ethiopia: Implication for impacting food security. Trop Anim Health Prod. 2010;42(3):341–347. DOI: 10.1007/s11250-009-9424-6
12. Adugna M, Asresie A. Physicochemical and microbiological quality of one-humped camel (*Camelus dromedarius*) milk: A Review. J Biol Agric Healthc. 2014;4(23):119-124. [available at]
13. Zahra HK, Ismail B, Wahiba, B. Physico-chemical analysis and microbiological quality of raw camel milk produced by Targui breed in Adrar region of Algeria. South Asian J Exp Biol. 2021;11(2):190-198. DOI: 10.38150/sajeb.11(2).p190-198
14. Mohamed AA, Hassan MM, Alsanie WF, Ibrahim AM, Rizk AM, Ismail M, Farid MA. Molecular diagnosis of mycoplasma species infection in camels using semi-nested PCR. Pak J Biol Sci. 2020;23(12):1506-1512. DOI: 10.3923/pjbs.2020.1506.1512
15. Gebru M, Tefera G, Dawo F, Tessema TS. Aerobic bacteriological studies on the respiratory tracts of apparently healthy and pneumonic camels (*Camelus dromedaries*) in selected districts of Afar region, Ethiopia. Trop Anim Health Prod. 2018;50(3):603-611. DOI: 10.1007/s11250-017-1476-4
16. Aqib AI, Muzammil I, Naseer MA, Shoaib M, Bakht P, Zaheer T, Yasir RK, Rabia LK, Muhammad U, Muhammad S, Qaisar T, Hafiz IH, Arslan S, Kashif P. Pathological insights into camel mastitis. Acta Trop. 2022;231(2):106415. DOI: 10.1016/j.actatropica.2022.106415
17. Alebie A, Molla A, Adugna W, Tesfaye A, Ejo M. Prevalence, isolation, identification, and risk factors of major bacterial cause of camel subclinical mastitis. Biomed Res Int. 2021;5522331:1-6. DOI: 10.1155/2021/5522331
18. Maunsell FP, Woolums AR, Francoz D, Rosenbusch RF, Step DL, Wilson DJ, Janzen ED. *Mycoplasma bovis* infections in cattle. J Vet Int Med. 2011;25(4):772-783. DOI: 10.1111/j.1939-1676.2011.0750.x
19. Sadoon AS. Clinical and subclinical mastitis in buffalue in Mosul area, Iraq. Iraq J Vet Sci. 2022;36(1):177-186. DOI: 10.33899/ijvs.2021.129644.1671
20. Rahawi AM, Al-Sabaawy HB, Ismail HK. Histopathological and histochemical study of mastitis in sheep. Iraq J Vet Sci. 2022;36(3):719-723. DOI: 10.33899/ijvs.2022.131595.1978
21. Al-Tofaily YK, Al Rodhan MN. Study on clinical mastitis (bacteriological) in she-camels (*Camelus dromedarius*) in some areas of middle Euphrates in Iraq. Al-Qadisiyah J Vet Med Sci. 2011;10(2):66-76. [available at]
22. Suheir IA, Salim MO, Yasin TE. Bacteria, mycoplasma, and fungi associated with subclinical mastitis in camel. Sudan J Vet Res. 2005;20,23-31. [available at]
23. Mederos-Iriarte LE, Poveda JB, Poveda CG, Vega-Orellana OM, Gutiérrez C, Corbera JA, Ramírez AS. Mycoplasma detection and isolation from one-humped camels (*Camelus dromedarius*). Trop Anim Health Prod. 2014;46(7):1317-1320. DOI: 10.1007/s11250-014-0639-9
24. Miyashita N, Akaike H, Teranishi H, Kawai Y, Ouchi K, Kato T, Hayashi T, Okimoto N. *Chlamydophila pneumoniae* serology: Cross-reaction with *Mycoplasma pneumonia* infection. J Infect Chemother. 2013;19(2):256-260. DOI: 10.1007/s10156-012-0494-4
25. Cimolai N, Bryan LE, To M, Woods DE. Immunological cross-reactivity of a *Mycoplasma pneumoniae* membrane-associated protein antigen with *Mycoplasma genitalium* and *Acholeplasma laidlawii*. J Clin Microbiol. 1987;25(11):2136-2139. DOI: 10.1128/jcm.25.11.2136-2139.1987
26. Tenk M, Bálint A, Stipkovits L, Bíró J, Dencső L. Detection of *Mycoplasma bovis* with an improved PCR assay. Acta Vet Hung. 2006;54(4):427-435. DOI: 10.1556/AVet.54.2006.4.1
27. Marouf S, Khalf MA, Alorabi M, El-Shehawi AM, El-Tahan AM, Abd El-Hack ME, Salem HM. *Mycoplasma gallisepticum*: A devastating organism for the poultry industry in Egypt. Poult Sci. 2022;101(3):101658-101668. DOI: 10.1016/j.psj.2021.101658
28. Fan HH, Kleven SH, Jackwood MW, Johansson KE, Pettersson B, Levisohn S. Species identification of avian mycoplasmas by polymerase chain reaction and restriction fragment length polymorphism analysis. Avian Dis. 1995;39:398–407. DOI: 10.2307/1591885
29. Daee AA, Khodakaram-Tafti A, Derakhshandeh A, Seyedin M. Identification of *Mycoplasma ovipneumoniae* and *Mycoplasma arginine* in sheep with pneumonia in north east of Iran. Iran J Vet Res. 2020;21(1):15-19. [available at]
30. Ulloa F, Soto JP, Kruze J, Mella A. Mycoplasma isolation in milk samples from dairy herds in Chile. Austral J Vet Sci. 2021;53(2):109-113. DOI: 10.4067/S0719-81322021000200109
31. Guo Y, Luo H, Guo S, Lei Y, Li Y, He S. Multi-locus sequence typing of *Mycoplasma bovis* to assess its genetic diversity from 2009 to 2018 in Ningxia Hui autonomous region, China. BMC Vet Res. 2020;16(1):1–9. DOI: 10.1186/s12917-020-02668-x
32. McCully MA, Brock KV. Development of a DNA hybridization probe for detection of *Mycoplasma bovis*. J Vet Diagn Invest. 1992;4(4):464-467. DOI: 10.1177/104063879200400420
33. Mohamed AA, Yassin MH, Hassan MM, Sabry AM, Ibrahim AM. Molecular and bacteriological diagnosis of *Mycoplasma* species infection in camels at Taif governorate, Saudi Arabia. Annu Res Rev Biol. 2018;23(5):1-6. DOI: 10.9734/ARRB/2018/39063

الكشف الجزيئي عن أنواع المفطورات من حليب الجمال

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الخلاصة

يكتسب الجمال العربي أهمية خاصة لما لها من استخدامات متعددة في النقل والوبر واللحوم وإنتاج الحليب. يعتبر حليب النوق غذاءً استثنائياً وذلك لاستخدامه في التغذية والعلاج. يؤثر التهاب الضرع بشكل كبير على إنتاج حليب النوق حيث يسبب خسائر اقتصادية كبيرة وخاصة عن\ما يكون التهاب الضرع بسبب الأحياء المجهرية ذات المنشأ الحيواني. هدف الدراسة الحالية إلى الكشف عن انتشار أنواع مختلفة من المفطورات وخاصة مفطورة بقرية في حليب الإبل باستخدام طريقة تفاعل البلمرة المتسلسل كأسلوب أساسي في التشخيص الجزيئي. جمعت خمسون عينة من حليب النوق المصابة بالتهاب الضرع تحت السرسري في العراق. أظهرت نتائج الدراسة الحالية أن ٢٦،٦٦% من عينات حليب النوق كانت موجبة للمفطورات. بينما بلغ معدل انتشار المفطورة البقرية في جميع العينات ٦١,٥٣%. خلصت الدراسة إلى أن المفطورات وتعد المفطورة البقرية أحد الجراثيم المسببة لالتهاب الضرع تحت السريري للإبل كما أن استخدام طريقة تفاعل البلمرة المتسلسل التي تعتبر أكثر سرعة وبساطة وحداثة في الكشف عن الجراثيم المفطورات في حليب الإبل.

34. Botes A, Peyrot BM, Olivier AJ, Burger WP, Bellstedt DU. Identification of three novel mycoplasma species from ostriches in south Africa. Vet Microbiol. 2005;111(3-4):159-69. DOI: 10.1016/j.vetmic.2005.10.017
35. Hamad MA, Al-Jumaa ZM, Al-Aalim AM, Mayahi MJ. Detection of *Mycoplasma bovis* in pneumonic calves. J Pure Appl Microbiol. 2019;13(4):2437-43. DOI: 10.22207/JPAM.13.4.59
36. Al-Salihi KA, Sahab A, Lifta A, Habib L. Epidemiological study of clinical and subclinical mastitis in she-camel in Samawah desert-Al Muthanna governorate. Mirror Res Vet Sci Anim. 2017;6(2):11-24. [available at]
37. Kaskous S. Prevalence of microbes in raw camel milk-an overview. IOSR J Agric Vet Sci. 2019;12:51-60. DOI: 10.9790/2380-1202015160
38. Zangerl P. Influence of udder health short textbook on milk science and milk hygiene. In: Krömker, V, editor. Microbiology of the products. Stuttgart: Medizinverlage Stuttgart GmbH & Co; 2007. 156-179 p.
39. Kaindi DM, Schelling E, Wangoh J, Imungi JK, Farah Z, Meile L. Microbiological quality of raw camel milk across the Kenyan market chain. Food. 2011;5(1):79-83. [available at]
40. Iyer AP, Albaik M, Baghallab I. Mastitis in camels in African and Middle East countries. J Bacteriol Parasitol. 2014;5(3):1. DOI: 10.4172/2155-9597.1000188
41. Abo Hashem M, Ibrahim S, Goda AS, Enany M. Diversity of microorganisms associated to she camels' subclinical and clinical mastitis in south Sinai, Egypt. Suez Canal Vet Med J. 2020;25(2):307-319. DOI: 10.21608/SCVMJ.2020.145315
42. Alamin MA, Alqurashi AM, Elsheikh AS, Yasin TE. Mastitis incidence and bacterial causative agents isolated from lactating she-camel (*Camelus dromedaries*). IOSR J Agric Vet Sci. 2013;2(3):7-10. DOI: 10.9790/2380-0230710
43. Sharma S, Citti C, Sagné E, Marenda MS, Markham PF, Browning GF. Correction: Development and host compatibility of plasmids for two important ruminant pathogens, *Mycoplasma bovis* and *Mycoplasma agalactiae*. Plos One. 2015;10(4):1-17. DOI: 10.1371/journal.pone.0119000
44. Burki S, Spergser J, Bodmer M, Pilo P. A dominant lineage of *Mycoplasma bovis* is associated with an increased number of severe mastitis cases in cattle. Vet Microbiol. 2016;196:63–6. DOI: 10.1016/j.vetmic.2016.10.016
45. Hashem YM, Mousa WS, Abdeen EE, Abdelkhalek HM, Nooruzzaman M, El-Askary A, Wareth G. Prevalence and molecular characterization of mycoplasma species, *Pasteurella multocida*, and *Staphylococcus aureus* isolated from calves with respiratory manifestations. Anim. 2022;12(3):312-324. DOI: 10.3390/ani12030312
46. Mahmoud MA, Wassif IM, El-Sayed AA, Awad WA, Farag HS, Noaman EA. Molecular identification of respiratory bovine mycoplasma isolated from Arabian camels in Egypt. J Egypt Vet Med Assoc. 2017;77(4):919-936. [available at]
47. Tigani-Asil E, Abdelwahab GE, Veedu JP, Khalafalla AI, Mohamed ZA, Ishag HA, Al Muhairi SM. Gangrenous mastitis in dromedary camels in UAE caused by *Streptococcus agalactiae*. BMC Vet Res. 2020;16(1):1-8. DOI: 10.1186/s12917-020-02382-8