

<http://www.pjbs.org>

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan



Research Article

Molecular Diagnosis of *Mycoplasma* Species Infection in Camels Using Semi-nested PCR

^{1,2}Alaa A. Mohamed, ^{1,3}Mohamed M. Hassan, ⁴Walaa F. Alsanie, ⁵Amena M. Ibrahim, ⁶Amira M. Rizk and ³Mohamed Ismail and ⁷Mona A. Farid

¹Department of Biology, Faculty of Science, Taif University, Taif, Saudi Arabia

²Department of Animal Reproduction and Artificial Insemination, Veterinary Research Division, National Research Center, Egypt

³Department of Genetics, Faculty of Agriculture, Menoufia University, Egypt

⁴Department of Clinical Laboratories, Faculty of Applied Medical Sciences, Taif University, Saudi Arabia

⁵Ismailia Animal Research Laboratory, Animal Health Research Institute, Agriculture Research Center, Egypt

⁶Department of Bacteriology, Immunology and Mycology, Faculty of Veterinary Medicine, Benha University, Egypt

⁷Department of Genetics, Faculty of Agriculture, Kafrelsheikh University, Kafr El-Sheikh, Egypt

Abstract

Background and Objective: Bacteriological isolation and identification of *Mycoplasma* species is difficult and time-consuming, therefore, molecular identification of *Mycoplasma* using PCR targeting specific genes is considered a specific and sensitive method for identification. The aim of current study was to isolate, characterize *Mycoplasma* infection in dromedary camels in Saudi Arabia. **Materials and Methods:** Nasal swabs were randomly collected from 93 camels and tested for *Mycoplasma* by sequencing of their 16S rRNA genes using universal primers. **Results:** The 93 samples, 24 were positive for *Mycoplasma*. However, no positive results were obtained using species-specific primers for *Mycoplasma arginine*, *M. bovis* or *M. mycoides* subsp. *mycoides*, thus, 16S rDNA sequencing methods and semi-nested PCR were employed. Sequences were matched to those in GenBank and phylogenetic analysis was performed. *Mycoplasma edwardsii* (77-84% similarity with *Mycoplasma edwardsii* ATCC 23462) and one isolate of *Mycoplasma yeastsii* (100% similarity with *M. yeastsii* GM274B) were identified. Further, some *Mycoplasma* species were identified as previously uncultured. The incidence of *Mycoplasma* infection in camels in Taif city, Saudi Arabia, was approximately 26%. **Conclusion:** This study provides insights into the accuracy and efficiency of PCR and universal primers for the detection and identification of *Mycoplasma*, thereby circumventing conventional culturing methods that require several days to complete and exhibit low accuracy.

Key words: Dromedary camel, *Mycoplasma*, 16S rDNA gene, sequencing, semi-nested PCR

Citation: Alaa A. Mohamed, Mohamed M. Hassan, Walaa F. Alsanie, Amena M. Ibrahim, Amira M. Rizk and Mohamed Ismail, 2020. Molecular diagnosis of *Mycoplasma* species infection in camels using semi-nested PCR. Pak. J. Biol. Sci., 23: 1506-1512.

Corresponding Author: Mohamed M. Hassan, Department of Biology, Faculty of Science, Taif University, Taif, Saudi Arabia

Copyright: © 2020 Alaa A. Mohamed *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Dromedary camels are one of the primary sources of income for a significant proportion of the population living in desert areas in the Kingdom of Saudi Arabia. Moreover, the meat and milk sourced from camels supplies approximately 30% of the total demand in the Kingdom of Saudi Arabia¹. Therefore, improving the overall health of domestic camels would help producers meet the recent increasing demand for high-quality camel meat and milk². Camels were previously considered to be resistant to most known animal diseases; however, recent studies reported that camels are susceptible to a variety of pathogens³.

Many advances have been made in the diagnosis and treatment of bacterial infections in camels over the past 2 decades, but the understanding of *Mycoplasma* infections in camels remains inadequate. The absence of information regarding the role of *Mycoplasma* as a causative agent in camel disease is primarily related to the scarcity of mollicute studies, including those on *Mycoplasma*, *Ureaplasma* or *Acholeplasma* in camels^{4,5}. Moreover, the primary habitat of *Mycoplasma* in clinically healthy camels remains unknown^{6,7}.

Mycoplasma belong to the class Mollicutes, which consist of wall-less prokaryotes that are among the smallest known self-replicating organisms^{8,9}. The absence of the bacterial cell wall in *Mycoplasma* species makes their infection difficult to diagnose and treat^{10,11}. *Mycoplasma*-related diseases are undetectable when diagnosis is based solely on clinical indicators such as pathological lesions or serological testing primarily because of the close taxonomic association of many *Mycoplasma* species. Classical methods used to detect and identify infection by *Mycoplasma* are time-consuming and can be complicated by serological cross-reactions between closely related taxa. The introduction of molecular diagnoses, including PCR methods have greatly improved the detection and identification of *Mycoplasma* infections^{11,12}. This study was conducted to determine the nature and type of *Mycoplasma* infection common in the upper respiratory tract of dromedary camels in Taif city, Mecca province, Kingdom of Saudi Arabia, using sequencing of *Mycoplasma* 16S rDNA, together with semi-nested PCR.

MATERIALS AND METHODS

Isolation of *Mycoplasma*: This study protocol was approved by the Taif University Medical Ethics Review Board and performed in accordance with the guidelines for the

protection of human and animal subjects. Nasal swabs and lung tissues were randomly collected from a total of 93 camels in a slaughter house in Taif, Saudi Arabia from October, 2017 to April, 2019, with a total number of 186 samples. The 93 swabs were cultured in pleuropneumonia-like organisms (PPLO) broth media at 37°C for 24 h and then inoculated on PPLO media agar plates at 37°C for 1 week, to evaluate the presence or absence of *Mycoplasma* colonies¹³. Lung tissue showing consolidation and grey and red hepatization were considered as abnormal. Lung tissues collected from slaughtered camels were packed in individual sterile plastic bags. Samples were transported in an icebox to the laboratory for storage at -20°C until analysis.

DNA extraction: Genomic DNA was extracted from the collected lung tissue using a Tissue DNA extraction kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. The DNA samples were stored at -20°C until further use.

PCR-based detection of *Mycoplasma* and amplification of the 16S rRNA gene: Paired primers were used for the molecular detection of *Mycoplasma* from nasal swab cultures. Three species-specific primers suitable for detecting three mollicute species and universal 16S rDNA primers capable of detecting all *Mycoplasma* species were employed (Table 1) according to Kazemiha *et al.*¹⁴. The universal 16S rRNA primer was designed as described by Lauerma *et al.*¹⁵ and was approximately 425 bp in length. These primers were used to target the conserved regions within 16S rDNA intergenic spacer regions. Thermal conditions were adjusted as described¹⁶. The semi-nested PCR primers described previously by Pettersson *et al.*¹⁷.

In vitro amplification of 16SrRNA gene by semi-nested PCR: The 16S rDNA genes from *Mycoplasma* were amplified by semi-nested PCR according to the methods of Pettersson *et al.*¹⁷. The semi-nested PCR primers were previously reported¹⁷. The PCR was performed using 2X PCR Go *Taq*[®] Green Master Mix (Promega). Each 25 µL reaction mixture contained all necessary reagents (200 nm of each dNTP and 0.6 units of *Taq* DNA polymerase). Fifty nanograms of genomic DNA and 10 pmol of each primer were added to the mix and amplification was performed as described by Persson *et al.*¹⁸. The presence of PCR products was confirmed by agarose (1.5% w/v) gel electrophoresis conducted at 100 V for 1 h.

Table 1: Oligonucleotide primer sequences used in the detection of *Mycoplasma* spp.

<i>Mycoplasma</i> species	Primer sequence 5'_____3'	Amplicon size (bp)	Annealing temperature (°C)
Universal primer	GTG GGG AGC AAA YAG GAT TAG A GGC ATG ATG ATT TGA CGT CRT	425	55
<i>M. arginini</i>	TGA TCA TTA GTC GGT GGA GAG TTC TAT CTC TAG AGT CCT CGA CAT GAC TC	326	60
<i>M. bovis</i>	TATTGGATCAACTGCTGGA-AGATGCTCCACTTATCTTAG	447	54
<i>M. mycoides</i> , <i>S. mycoides</i>	TAGAGGTACTTTAGAT GATATCAAAGGTGATGGT	1500	50

Sequencing of 16S rRNA genes: The 16S rDNA fragments measuring approximately 425 bp were purified using the QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions and sequenced using the same primers and the gene analyzer 3121 sequencer. This step was performed by Macrogen Co. (Seoul, Korea). The *Mycoplasma* 16S rDNA obtained sequences were then aligned with known 16S rDNA sequences deposited in GenBank using the basic local alignment search tool of the National Center for Biotechnology Information and percent homology scores were generated to identify *Mycoplasma* species. The identified sequences were aligned using Molecular Evolutionary Genetics Analysis (MEGA) version 7.10 software and a phylogenetic tree was produced¹⁹.

RESULTS

Identification by PCR analysis: Ninety three samples were tested for *Mycoplasma* infection using specific primers for (*Mycoplasma arginine*, *M. bovis* and *M. mycoides* subsp. *mycoides*), but, not obtained positive results for each species. Then, 24 mycoplasma isolates were obtained by PCR detection using universal 16S rDNA and sequenced. This resulted in the identification of specific isolates, including *M. edwardii* and *M. yeastsii*. Specifically, isolates Myco 1, 3, 4, 6, 9, 10, 11, 12, 13, 14 and 15 were identified as *Mycoplasma edwardii*, exhibiting 77-84% similarity with *Mycoplasma edwardii* ATCC 23462. Similarly, isolate Myco 16 was identified as *M. yeastsii*, exhibiting 100% similarity to *M. yeastsii* GM274B. In contrast, other isolates were identified as uncultured *Mycoplasma* in the GenBank database (Fig. 1).

In vitro semi-nested PCR: To perform semi-nested PCR using U1/U8 degenerate primers, 1.5 kb fragment representing the 16S rDNA from *Mycoplasma* isolates was amplified using U1 and U8. The PCR yielded unsatisfactory results, therefore, PCR temperature profiles in addition to the amount and concentration of U1 and U8 primers were adjusted

accordingly. The amount and concentration of U1 and U8 primers. This non-specific melting curve reflects the formation of primer dimers in the absence of target DNA, likely because of both the high primer concentrations and their degeneracy. While primer dimers were detected in association with amplicons in most *Mycoplasma* strain samples and in the positive control (as indicated by a sharp band identical to that observed in the gel doc measuring 1500 bp), this band was not observed in lane 2 (Fig. 2). Two other samples did not yield amplicons. As the semi-nested PCR assay was performed using the recommended set of U1/U8 primers for *Mycoplasma* identification, the relative sensitivity of the PCR and the semi-nested PCR assay when using the optimized PCR parameters was evaluated, by testing the serial dilution of the supernatant from a cell contaminated with a known *Mycoplasma*. The method was validated by PCR and visualization of the resulting PCR product was accomplished by electrophoresis in an agarose gel (Fig. 3). The semi-nested PCR assay was performed using the U2/U5 primer set showing a sharp band that was identical to those observed in the positive control (900 bp) (Fig. 4, lane 2). Accordingly, in this method, the lowest dilution of genomic DNA detectable was successfully estimated to accurately produce amplicons. The 16S rDNA sequences of mobile *Mycoplasma* strains deposited in GenBank were identical to those found in the current study. Ribosomal operons are essential in understating the evolution of *Mycoplasma*. Additionally, 16S rDNA sequences has been extensively used to reconstruct phylogenetic relationships among different *Mycoplasma* species. Upon using a semi-nested PCR assay utilizing the new combination of U2/U8, a sharp band was obtained which is identical to the positive control of 1250 bp. In this assay a primer pair that is complementary to the universal regions U2 and U8, yielded 900 and 1250 bp PCR products, respectively (Fig. 5) with an overlapping region of approximately 650 bp was used.

The semi-nested PCR relies on the interference of PCR with the first primer pair complementing the global regions U1 and U8. The resulting product is approximate 1500 bp which can be readily used in amplification of 95%

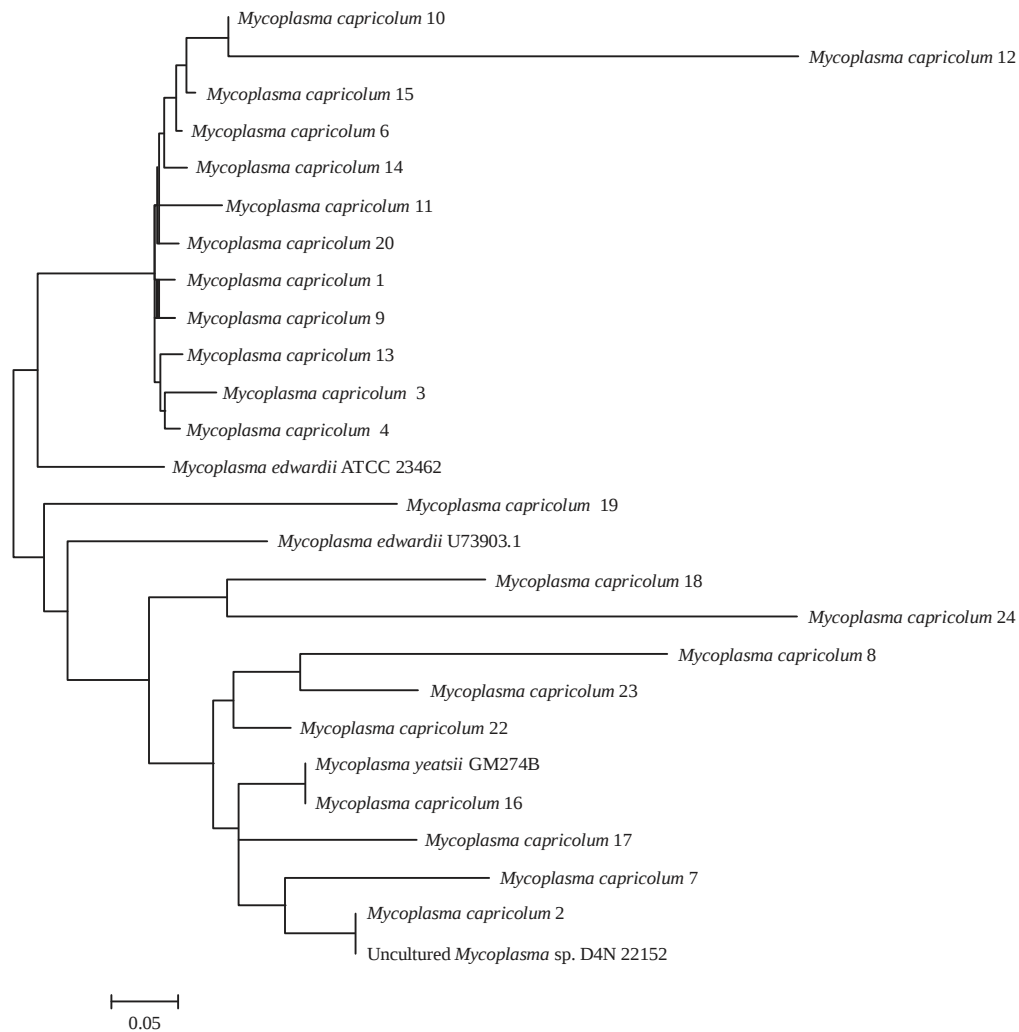


Fig. 1: Phylogenetic tree based on 16S rRNA sequences of *Mycoplasma* species and related species

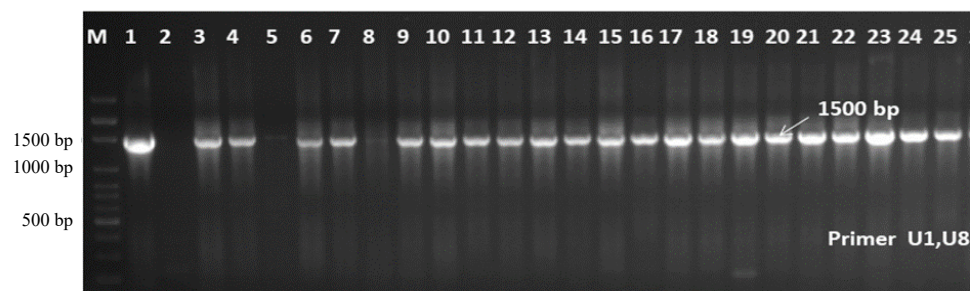


Fig. 2: Amplification of PCR products from mycoplasma isolates using primers U1 and U8

Lane 1: Positive control and Lanes 2-25: Sample isolates, approximate size of the amplicons was 1500 bp

of the genes. The amplicon is then diluted and amplified again in two independent semi-nested PCR cycles with one primer pair complementary to the universal regions U1 and U5 and the other complementary to the universal regions U2 and U8. These nested -PCR reactions produce 650,

900 and 1250 bp, respectively. Thus, it can be clarified that the three outcomes of chain polymers reactions give sequences of specific lengths using the eight primers mentioned through which the species of mycoplasma can be defined.

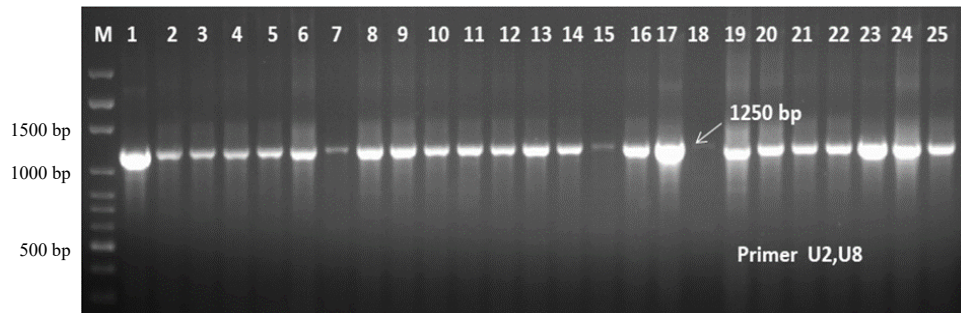


Fig. 3: Amplification of PCR products from mycoplasma isolates using primers U2 and U8
Lane 1: Positive control and Lanes 2-25: PCR products, approximate size of the amplicon was 1250 bp

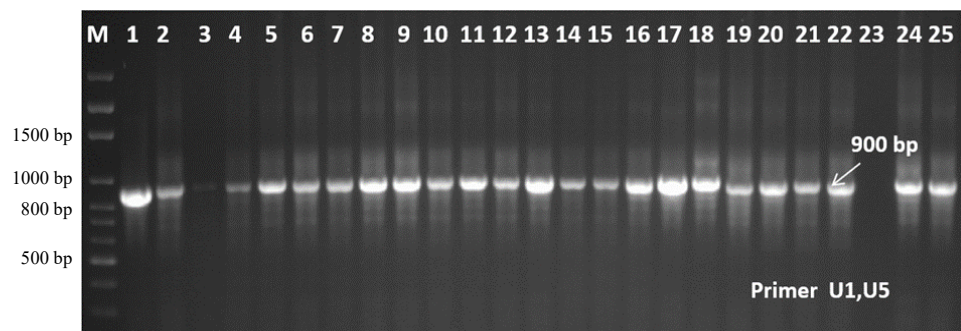


Fig. 4: Amplification of PCR products from mycoplasma isolates using primers U1 and U5
Lane 1: Positive control and Lanes 2-25: Sample isolates, approximate size of the amplicons was 900 bp

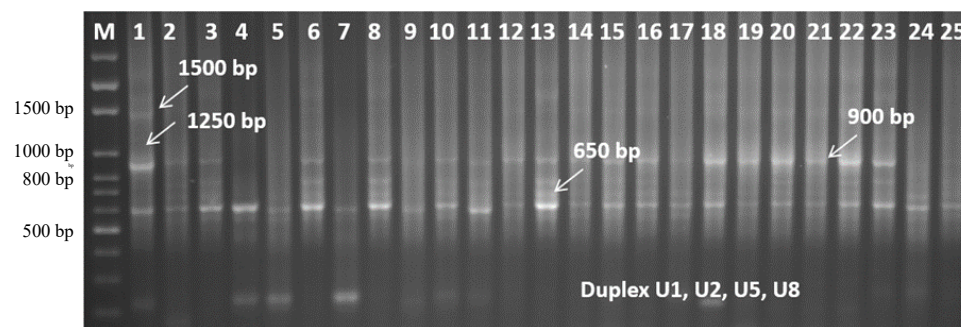


Fig. 5: Amplification of universal primer regions U1, U2, U5 and U8 of mycoplasma isolates with deferent amplicon sizes
Lane 1: Positive control and Lanes 2-25: U1, U2, U5 and U8, approximate sizes of the amplicons were 1500, 1250, 900 and 650 bp, respectively

DISCUSSION

Bacteriological isolation and identification of *Mycoplasma* species is difficult, insensitive and costly^{20,21}. Bacteriological isolation of 93 lung tissues collected from diseased or apparently healthy camels were unable to identify *Mycoplasma* isolates in any of the samples. The failure to obtain positive results using these traditional methods agreed with the findings of previous studies^{20,21}. Those authors also found that the low isolation percentage of *Mycoplasma* identified was attributed to the presence of toxic or inhibitory

substances in the camels' tissue, preventing cell culture and to the fastidious nature of many pathogenic *Mycoplasma* species. Using PCR in tandem with universal primers, 24 of the 93 samples tested positive for *Mycoplasma* infection. All 24 *Mycoplasma* isolates were molecularly identified at the genus level using PCR by detecting the 16S rRNA gene. The 16S rDNA sequences will likely play an important role in classifying *Mycoplasma* as more species are described²². It was used primers specific to 3 *Mycoplasma* species (*M. arginine*, *M. bovis* and *M. mycoides* subsp. *mycoides*), however, no positive results were obtained. It was identified specific

isolates, including *M. edwardii* and *M. yeastsii*. Specifically, isolates were identified as similar to *M. edwardii* ATCC 23462 and *M. yeastsii* GM274B. Traditional identification methods (morphological methods) provide significantly less diagnostic information compared to PCR-based methods. The number of sequences of the ribosome gene from mycoplasma deposited in GenBank is approximately one hundred genetic sequences, thus the genetic sites and genetic affinity of the new mycoplasma species can be determined quickly.

PCR primers that complement the global regions of the 16S rRNA genes can be designed from Mycoplasma and such primers can be used in the polymerase chain reaction of genes such as the mycoplasma gene^{9,14,23}.

Then it is used to determine the sequence of such genes, which can be compared with the genetic databases of mycoplasma, and to determine the ratio of genetic affinity and the precise definition of the different genera. In addition, it can be determined and precisely whether these types of mycoplasmas have been determined by the row before or not^{24,25}.

As is known so far, approximately 100 16S rRNA Mycoplasma has been deposited in databases, allowing the production of genetic trees with genetic affinity that allow precise identification of the type and gender of mycoplasmas and these databases are the source and reference in the molecular definition of such genera of mycoplasma. The 16S rRNA genes sequencing provide a powerful tool in *Mycoplasma* classification^{26,27}. These primers are primarily designed for the study of Mycoplasma, but can also be used to amplify and sequence 16S rRNA genes for many bacterial species related to mycoplasma. Methods of designing a polymerase chain reaction (PCR), especially this semi-nested PCR, have been discussed in addition to the possibility of applying this unique reaction of a high-specific type and speed with many bacterial species²⁸. The data of this study demonstrated that the incidence of mycoplasmas in camels in Taif city was approximately 26%. Further, these findings indicated that PCR sequencing using universal primers is an accurate and time-efficient method to detect and identify mycoplasmas. This method overcomes the issues commonly associated with conventional culture methods used in to identify mycoplasmas.

CONCLUSION

The current study indicates that, the importance of using nested PCR and 16S rDNA sequencing technology for diagnosis of camel's *Mycoplasma* infection, the nested PCR technology is specific molecular technology for *Mycoplasma*

identification, more sensitive test. These techniques are simple and quick methods of detection and isolate infected animals, so it is a way to reducing economic losses in animal industry.

SIGNIFICANCE STATEMENT

Camels are susceptible to various pathogens including mollicute bacteria such as *Mycoplasma*. Few studies have addressed *Mycoplasma* infection in camels and *Mycoplasma* are difficult to detect. Here, we identify known and previously undescribed *Mycoplasma* from camel nasal tracts and demonstrate an effective method for doing so.

REFERENCES

1. Faye, B., 2015. Role, distribution and perspective of camel breeding in the third millennium economies. Emir. J. Food Agric., 27: 318-327.
2. Abdallah, H.R. and B. Faye, 2013. Typology of camel farming system in Saudi Arabia. Emir. J. Food Agric., 25: 250-260.
3. Faye, B., H. Madani and A.B. El-Rouili, 2014. Camel milk value chain in Northern Saudi Arabia. Emir. J. Food Agric., 26: 359-365.
4. El-Metwally, A.E., M.H. Yassin and A. El-Hallawany, 2010. Mycoplasmosis in slaughtered she-camels: Pathological studies. Global Vet., 4: 474-482.
5. Durrani, A.Z., Z. Bashir, I. Rasheed and N.A. Sarwar, 2017. Epidemiological study of common diseases and their risk factors in camels in South Punjab, Pakistan. Microb. Pathogen., 108: 6-12.
6. Elfaki, M.G., B. Abbas, O.M. Mahmoud and S.H. Kleven, 2002. Isolation and characterization of *Mycoplasma arginini* from camels (*Camelus dromedaries*) with pneumonia. Compa. Immunol. Microbiol. Infect. Dis., 25: 49-57.
7. Kheirabadi, K.H.P. and A. Ebrahimi, 2007. Investigation of *Mycoplasma agalactiae* in milk and conjunctival swab samples from sheep flocks in West central, Iran. Pak. J. Biol. Sci., 10: 1346-1348.
8. Brown, D.R., M. May, J.M. Bradbury and K.E. Johansson, 2010. Class I. Mollicutes Edward and Freundt 1967, 267^{AL}. In: Bergey's Manual of Systematic Bacteriology, Vol. 4, Krieg, N.R. and J.T. Staley (Eds.), Springer-Verlag, New York, pp: 568-573.
9. Mohamed, A.A., M.H. Yassin, M.M. Hassan, A.M. Sabry and A.M. Ibrahim, 2018. Molecular and bacteriological diagnosis of *Mycoplasma* species infection in camels at Taif Governorate, Saudi Arabia. Annu. Res. Rev. Biol., 23: 1-6.
10. Sobouti, B., S. Fallah, M. Mobayen, S. Noorbakhsh and Y. Ghavami, 2014. Colonization of *Mycoplasma hominis* and *Ureaplasma urealyticum* in pregnant women and their transmission to offspring. Iran. J. Microbiol., 6: 219-224.

11. Qasem, J.A., S.A. Al-Mouqati, E.M. Al-Ali and A. Ben-Haji, 2015. Application of molecular and serological methods for rapid detection of *Mycoplasma gallisepticum* infection (Avian mycoplasmosis). Pak. J. Biol. Sci., 18: 81-87.
12. Abo-Elnaga, T.R., A.S. Gouda, Y.M. Kamel, M.A. Mahmoud and W.A. Osman, 2012. Application of the polymerase chain reaction for identification of *Mycoplasma* isolated from aborted She Camel. Global Vet., 8: 386-392.
13. Evans, J.D., S.L. Branton and S.A. Leigh, 2009. Effect of dosage and vaccination route on transmission of a live attenuated *Mycoplasma gallisepticum* vaccine: A broiler model. Avian Dis., 53: 416-420.
14. Kazemiha, V.M., M.A. Shokrgozar, M.R. Arabestani, M.S. Moghadam and S. Azari *et al.*, 2009. PCR-based detection and eradication of mycoplasmal infections from various mammalian cell lines: A local experience. Cytotechnology, 61: 117-124.
15. Lauerma, L.H., A.R. Chilina, A.R. Closser and D. Johansen, 1995. Avian mycoplasma identification using polymerase chain reaction amplicon and restriction fragment length polymorphism analysis. Avian Dis., 39: 804-811.
16. Mohamed, R.A. and E.B. Abd El-Salam, 2008. A review on pneumonic pasteurellosis (Respiratory Mannheimiosis) with emphasis on pathogenesis, virulence mechanisms and predisposing factors. Bulg. J. Vet. Med., 11: 139-160.
17. Pettersson, B., T. Leitner, M. Ronaghi, G. Bölske, M. Uhlen and K.E. Johansson, 1996. Phylogeny of the *Mycoplasma mycoides* cluster as determined by sequence analysis of the 16S rRNA genes from the two rRNA operons. J. Bacteriol., 178: 4131-4142.
18. Persson, A., B. Pettersson, G. Bölske and K.E. Johansson, 1999. Diagnosis of contagious bovine pleuropneumonia by PCR-Laser-induced fluorescence and PCR-Restriction endonuclease analysis based on the 16S rRNA genes of *Mycoplasma mycoides* subsp. *mycoides* SC. J. Clin. Microbiol., 37: 3815-3821.
19. Kumar, S., G. Stecher and K. Tamura, 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol. Biol. Evolut., 33: 1870-1874.
20. Atalla, H., I. Lysnyansky, Y. Raviv and S. Rottem, 2015. *Mycoplasma gallisepticum* inactivated by targeting the hydrophobic domain of the membrane preserves surface lipoproteins and induces a strong immune response. Plos One, Vol. 10. 10.1371/journal.pone.0120462.
21. McAuliffe, L., B. Kokotovic, R.D. Ayling and R.A.J. Nicholas, 2004. Molecular epidemiological analysis of *Mycoplasma bovis* isolates from the United Kingdom shows two genetically distinct clusters. J. Clin. Microbiol., 42: 4556-4565.
22. Farid, M.A., A.A. Abo-Shosha, E.S.B. Belal and M.M. Hassan, 2018. Genotyping of pathogenic *Mycoplasma bovis* isolated from cattle in Kafr El-Sheikh province, Egypt. J. Pure Applied Microbiol., 12: 2103-2110.
23. Das, S., H.R. Dash, N. Mangwani, J. Chakraborty and S. Kumari, 2014. Understanding molecular identification and polyphasic taxonomic approaches for genetic relatedness and phylogenetic relationships of microorganisms. J. Microbiol. Methods, 103: 80-100.
24. Pettersson, B., G. Bolske, F. Thiaucourt, M. Uhlen and K.E. Johansson, 1998. Molecular evolution of *Mycoplasma capricolum* subsp. *capripneumoniae* strains, based on polymorphisms in the 16S rRNA genes. J. Bacteriol., 180: 2350-2358.
25. Pettersson, B., M. Uhlen and K.E. Johansson, 1996. Phylogeny of some mycoplasmas from ruminants based on 16S rRNA sequences and definition of a new cluster within the hominis group. Int. J. Syst. Evolut. Microbiol., 46: 1093-1098.
26. Pettersson, B., K.E. Johansson and M. Uhlen, 1994. Sequence analysis of 16s rRNA from mycoplasmas by direct solid-phase DNA sequencing. Applied Environ. Microbiol., 60: 2456-2461.
27. Yassin, M.H., A.A. Mohamed, M.M. Hassan, A.A.E.A. Baiomy and A.M. Ibrahim, 2018. Molecular characterization of two new *Mycoplasma* species isolated from chickens in Saudi Arabia. Biotechnology, 17: 142-150.
28. Fischer, M.A., S. Güllert, S.C. Neulinger, W.R. Streit and R.A. Schmitz, 2016. Evaluation of 16S rRNA gene primer pairs for monitoring microbial community structures showed high reproducibility within and low comparability between datasets generated with multiple archaeal and bacterial primer pairs. Front. Microbiol., Vol. 7. 10.3389/fmicb.2016.01297.