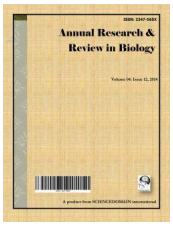

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Molecular and Bacteriological Diagnosis of *Mycoplasma* Species Infection in Camels at Taif Governorate, Saudi Arabia

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Authors' contributions

This work was carried out in collaboration between all authors. Author AAM designed the study, wrote the protocol, helped in molecular part of work and wrote the first draft of the manuscript. Author MHY wrote the protocol, cultured the samples and follow up Mycolpasma culture. Author MMH wrote the protocol, worked with the molecular identification, managed the analysis of study. Author AMS helped in manage the analyses of study and managed the literature searches. Author AMI designed the study and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: We designed this work to confirm if the PCR technique is more rapid and specific than traditional diagnostic method by culture.
Study Design: *In vitro* experimental and molecular study.
Place and Duration of Study: Genetic engineering and biotechnology unit, Taif University, Saudi Arabia from October, 2016 to September, 2017.
Methodology: Ninety three nasal and tracheal swabs and lung samples were collected from camel in Taif slaughterhouse, Saudi Arabia. All samples were tested by culture and PCR method using universal primer of 16S rRNA gene.
Results: There was no positive result obtained by culture method, but 30 (32.2%) of nasal swabs were positive using PCR method. Moreover, we used species-specific primers for *Mycoplasma arginine, M. bovis* and *M. mycoides subspecies mycoides* to identify the isolates at species level, but no positive results obtained with specific primers. These positive samples could be other *Mycoplasma* species.
Conclusion: These results indicate that PCR technique is a specific molecular detection technique for *Mycoplasma* identification, and more sensitive test. These techniques are simple and fast methods to detect and isolate infected animals.

Keywords: Mycoplasma species; 16S rRNA gene; PCR technique; camels.

1. INTRODUCTION

The one-humped camel (*Camelus dromedarius*), often referred as the Arabian camel, It is one of two species within the genus *Camelus* [1]. Camels, compared to other livestock in the same locations, have been reported to be less susceptible to many diseases such Mycoplasmosis in camels [2]. Mollicutes comprise a group of wall-less prokaryotes and are among the smallest self-replicating organisms [1]. Elfaki et al. [3] isolated *Mycoplasma arginini* from pneumonic lesions in camel with 6% isolation. The *Mycoplasma* forms a constant source of infection for young animals that are more susceptible to developing clinical symptoms [4]. Consequently the pathogen cannot be detected during the incubation period. Moreover, the serological cross reactions among the *Mycoplasma* species are a critical problem [5,6]. In the absence of effective antibiotic or vaccination the only strategy currently available to control infection is the strict segregation of *Mycoplasma* infected animals from healthy herd. The classical methods for detecting and identifying *Mycoplasma* are time consuming and complicated by serological cross-reactions between related organisms [6,4]. Additional problems can be caused by bacterial contamination of samples, as this usually prevents *Mycoplasma* growth [6]. In view of these difficulties simpler, faster, and less hazardous and usually more sensitive and specific diagnostic methods are needed for detection of this organism [4]. Polymerase chain reaction (PCR) can yield rapid and specific diagnosis of infections caused by *Mycoplasma* [7,4]. PCR with specific primers have better chance for the detection of *Mycoplasma* species in both early and chronic infections [8]. In camels, birthing rate rarely exceed 40% in nomadic herds and 70% in more intensive herds [4]. In addition to low birthing rates, camel herds suffer from high neonatal loss sometimes reaching epizootic proportions [9]. The studies on the incidence and etiology of abortion in camelidae are scarce [10,4] and little is known about the role of *Mycoplasma* in the etiology of diseases in camels. This is partially due to the lack of investigation on the occurrence of mollicutes such as *Mycoplasma*, *Ureaplasma* or *Acholeplasma* in camels. Moreover, little data are available on the *Mycoplasma* flora of clinically healthy camels [3]. So the goal of this study was to throw out the light on *Mycoplasma* as a probable cause of many diseases in camel using PCR as a specific, sensitive and rapid technique for early diagnosis with comparison with culture method.

2. MATERIALS AND METHODS

2.1 Isolation of *Mycoplasma*

The lung samples and swabs from nose and trachea were randomly collected from a total of 93 camels in Taif slaughterhouse, Saudi Arabia from October, 2016 to September, 2017. The samples and swabs were cultured in pleuropneumonia-like organisms (PPLO) broth

media at 37°C for 24 h., and then inoculated in PPLO media agar plate at 37°C for 1 week to examine the presence of *Mycoplasma* colonies [11].

2.2 Isolation of *Ureaplasma*

A serial tenfold dilution of original samples was done in *Ureaplasma* broth medium. Original and diluted samples were incubated aerobically at 37°C when the color changed from yellow to red without turbidity, the changed broth was sub-cultured on *Ureaplasma* agar plates as soon as possible, agar plates were incubated under 10% CO_2 tension. The incubated plates were examined microscopically for the characteristic *Ureaplasma* colonies [12].

2.3 DNA Extraction

Genomic DNA was extracted from the *Mycoplasma* sample by using bacterial DNA extraction kit (Promega, USA), according to the manufacturer's instructions. DNA samples were stored at -20°C until used. The laboratory work was performed in the Biotechnology and Genetic Engineering Unit and Scientific Research Deanship, Taif University Taif, KSA.

2.4 Oligonucleotides and PCR-based Detection of *Mycoplasmas*

A total of three species-specific primers suitable for the detection of three mollicutes, as well as a universal generic-specific primer capable of detecting all *Mycoplasma* species (listed in Table 1) according to Kazemiha et al. [13], were used to target the conserved region of 16S rDNA intergenic spacer regions. The universal primers were degenerate having equal quantities of T and C nucleotides at position 13 (Y) of the sense and A and G nucleotides at position 20 (R) of the anti-sense primers, respectively. PCR reaction mixtures were prepared in a total volume of 25 µL containing, 19 µL PCR buffer, 50 mM each of dNTP, 15 pmol of each primer, 1 U Taq DNA polymerase, 1.5 mM $MgCl_2$, and 2 µl mycoplasmal genomic DNA as template. Thermal profiles were as follows: initial denaturation at 94°C for 5 min, 35 cycles consisting of denaturation at 94°C for 60 s, annealing at 60°C for 30 s and extension at 72°C for 60 s, followed by a final extension at 72°C for 5 min. The annealing temperature of the universal primers was set at 55°C. The PCR products were analyzed on 1.5% (w/v) agarose gel. DNA fragments were visualized with a UV transilluminator after being stained with ethidium bromide and visualized by UV illumination and were photographed by a Bio-Rad Gel Doc 2000 device.

3. RESULTS AND DISCUSSION

3.1 Culture Methods

The study was conducted in two stages. Traditional culture method and PCR method to identify *Mycoplasma* colonies. Initially, a total of 93 samples were taken from different organs of Camels (Table 2). With culture method, *Mycoplasma* isolation was not achieved from any of these samples in a PPLO broth and/or solid culture media. *Ureaplasma* was also tested in *Ureaplasma* broth and Agar medium, but *Ureaplasma* was not isolated from any of these samples. Traditionally, culture method was used to isolate *Mycoplasma* colonies on agar medium, but there was no positive mycoplasma isolate obtained with this method [14,15].

3.2 PCR Method

Mycoplasmal DNA was extracted from each of the swabs. We used PCR with universal primers [16,17], for identification of *Mycoplasma* in cultures. Thirty (30) out of the 93 (32.2%) samples tested with universal primers were

Table 1. The sequences of oligonucleotide primers used for detection of *Mycoplasma spp*.

***Mycoplasma* spp.**	**Primer sequence 5`→ 3`**	**Amplicon size**	**Annealing temp.**
Universal primer	GTGGGGAGCAAAYAGGATTAGA GGCATGATGATTTGACGTCRT	425 bp	55°C
M. arginini	TGATCATTAGTCGGTGGAGAGTTC TATCTCTAGAGTCCTCGACATGACTC	326 bp	60°C
M. bovis	TATTGGATCAACTGCTGGA- AGATGCTCCACTTATCTTAG	447 bp	54°C
M. mycoides s. mycoides	TAGAGGTACTTTAGAT GATATCAAAGGTGATGGT	1500bp	50°C

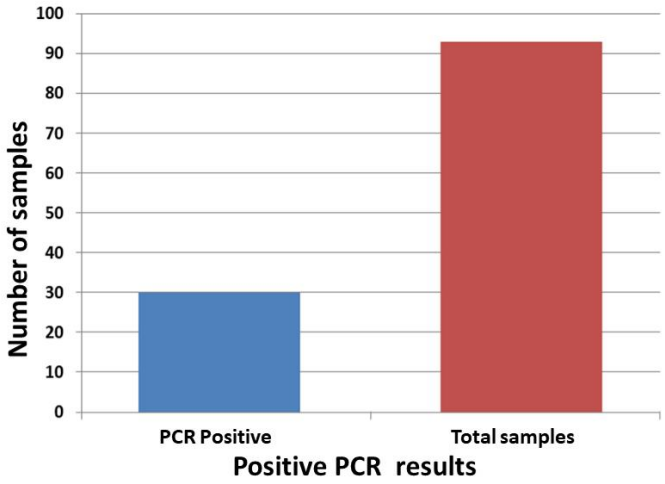

Fig. 1. Number of positive using PCR and total samples

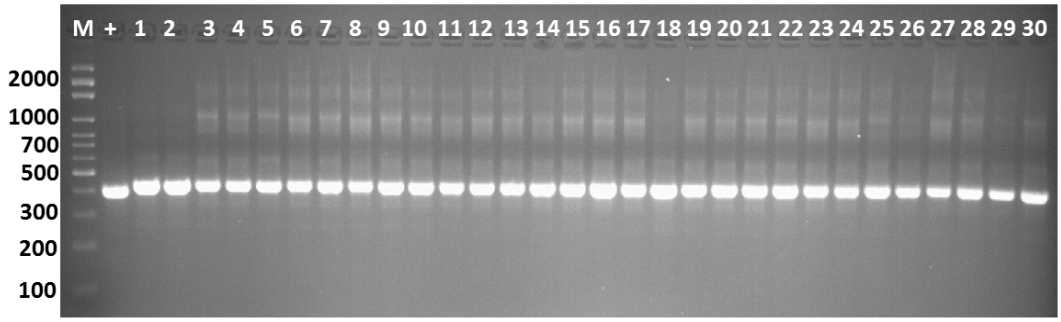

Fig. 2. Analysis of PCR products from thirty isolates obtained by amplification with universal primer of 16S rDNA. Molecular-size was applied from lane 2 to 31 and a positive control in lane 1. The approximate sizes of the amplicons are given in 425 bp

positive (Fig. 1). The 16S rDNA PCR amplicon from the thirty positive samples obtained,showed that they had the same length (425 bp) as *Mycoplasma* species (Fig. 2). This is in agreement with findings of other researchers who showed that PCR provides a rapid diagnosis and identification of *Mycoplasma*, and also showed that PCR assay has several features that simplify the diagnosis of *Mycoplasma* infections [17]. To increase sensitivity of *Mycoplasma* detection, PCR based on specific sequences of nucleotides has been used (Atalla et al. [14]. Multiplex PCR, also developed for *Mycolpasma* detection and identification [17,14]. Moreover, we used species-specific primers for three *Mycoplasma* species (*Mycoplasma arginine, M. bovis* and *M. mycoides subspecies mycoides*) to identify the isolates at species level, but no positive results obtained with the species-specific primers.

Table 2. Nasal and tracheal swabs collected from camels in Taif

Camel description	Type of specimens	No. of samples collected
healthy camels	Nasal swabs	93
healthy camels	Tracheal swabs	93

Mycoplasma is difficult to culture and diagnosis usually relies on serology in the past [18]. However, serology is not sufficiently rapid and reliable especially in specificity and is usually positive at about 7 days after the onset of disease [19,20]. The PCR technology that has been used for diagnosing Mycoplasma infections has several limitations, *e.g*. (*i*) PCR inhibitors in samples can lead to false-negative results; (*ii*) contamination can easily result in false-positive;

(*iii*) acquiring good samples are relatively difficult; and (*iv*) the time point for sampling influences results. It was reported that the diagnostic accuracy of PCR may decrease at ≥ 7 days after onset of disease in contrast to serology [21]. Different diagnostic studies in this field have generated inconsistent diagnostic accuracy due to differences in threshold, test methods and PCR types. Because of various confounding factors, the exact diagnostic accuracy of PCR for *Mycoplasma* species is difficult to establish. Results from this meta-analysis indicate that commercial PCR tests generated consistent results with high specificity, but sensitivity estimates were lower and more variable than specificity. Potential explanations for these variations may include the different types of PCR and reference standard, the types of subjects, the time point for sampling and the qualities of different samples, the standard control of PCR and threshold, etc [17,4].

4. CONCLUSION

In conclusion, the present study suggest the importance of using PCR technique in diagnosing Mycoplasma infections with advantages, PCR technique is a specific molecular technique for *Mycoplasma* identification, and more sensitive test. These techniques are simple and fast methods to detect and isolate infected animals, so it is a way to decrease economic losses in animal breeding.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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