Original Article

Detection of *Mycoplasma capricolum capricolum* from goats of Qom province, Iran

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ABSTRACT

*Mycoplasma capricolum subsp capricolum* (Mcc) is one of the etiological agents of contagious agalactia (C.A) in goats which can cause significant economic losses. The aim of this study was to detect Mcc from goats of Qom province in Iran. A total of 111 samples were collected from suspected goats to C.A and cultured in PPLO broth supplemented for Mcc isolation. The bacteria DNAs were extracted from clinical samples and the PCR assay was performed to detect *Mycoplasma* genus, *cluster Mycoides* and *Mcc* from culture. Out of the 111 samples, 33 (29.7%) sample cultures were shown positive and typical *Mycoplasma* colonies in PPLO agar culture method, 53 (47.7%) samples were scored positive by *Mycoplasma* genus PCR, 8 (7%) of the samples were scored positive by using *mycoides cluster* PCR and finally 2 (1.8%) of the samples were scored positive by using *Mcc* PCR method. The result of this study showed that *Mcc* was detected for the first time from Qom province in Iran. Therefore, Qom could be one of the geographical distribution and efficient factors of *Mcc* in Iranian goats. The lung samples and lymph nodes also could be significant samples for detection of *Mcc*.

Keywords: *Mycoplasma capricolum subsp capricolum*, Culture, PCR, Goats, Qom province, Iran

INTRODUCTION

Contagious agalactia (C.A) is one of the serious diseases to affect small ruminants on all five continents (Lambert *et al* 1987, DaMassaa *et al* 1992, Bergonier *et al* 1997). It is known for nearly 200 years. It is primarily a disease of dairy sheep and goats, characterized by mastitis, arthritis and keratoconjunctivitis (OIE 2008). *Mycoplasma agalactiae* (*M. agalactiae*) is often considered the classical agent of the C.A (Nicholas *et al* 2008) but recent years *Mycoplasma mycoides subsp mycoides LC* (large colony), *Mycoplasma capricolum subsp capricolum*(*Mcc*), *Mycoplasma mycoides subsp Capri* (*Mmc*) and *Mycoplasma putrefaciens* (*M. putrefaciens*) have been isolated with similar clinical signs of C.A from goats (Nicolass *et al* 2008, Corrales *et al* 2008, Bergnioer *et al* 1997). These pathogens are the members of the *Mycoplasma mycoides cluster* (Woubit *et al* 2007) and also they are rapidly mentioned as causative agents of the C.A (OIE 2008, Corrales *et al* 2008, Lambert 1988). *Mcc* principally affects goat, however it could occur in sheep, wild
goats and cattle (Bergnioer et al 1997) as well. Tully et al. 1974 named it \textit{capricolum} Mycoplasma capricolum. It is widely distributed and highly pathogenic, particularly in North Africa and Australia (Nicolass et al 2008, Cottew et al 1982), but frequency of occurrence is low (OIE 2008). Mcc causes sporadic outbreaks of caprine arthritis, or polyarthritis, mastitis, keratoconjunctivitis, pneumonia, septicemia and vulvovaginitis (Nicholass et al 2008, Corrales et al 2008, Bergnioer et al 1997). The young animals are the main host of this pathogen for development of the disease. Mcc could be detected from respiratory systems, mammary glands, joint, genital system and nervous system in young animals (Corrales et al 2008, Bergnioer et al 1997). De la Fa et al. 2007 isolated Mcc for the first time on the island of Lanzarote in Spain. Monnerat et al. 1999 could distinguish between Mcc and Mycoplasma capricolum subsp caprineumoniae (Mccp) based on LppA genes. Vaccination strategies against C.A of sheep and goats were based on both live attenuated or inactive vaccines (Foggie et al 1971a, Foggie et al 1971b, Madanat et al 1991) then the efficacy of those vaccines were evaluated (Lambert et al 1989, Hasso et al 1993). In Iran, Antigens for vaccination were prepared from M. agalactiae. Live vaccines from attenuated M. agalactiae cultures are more effective than inactivated vaccines, but their use is not permitted in all countries which are affected by C.A. (Madanat et al 1991).

While Iran is one of the biggest countries in breeding and maintenance of goats and sheep in the world, there is a only inadequate information available on the infection diseases’ status of these animals in the present country. Detection and prevalence of \textit{M. agalactiae} on sheep and goats in different provinces of Iran were determined (Abtin et al 2013, Kheyrkhah et al 2011, Moradi Bidehendi et al 2011). However, no investigation in to the isolation and detection of Mcc has been conducted in Iran. The aim of this study was to detect Mcc from clinical samples suspected to C.A in goats of Qom province, Iran.

**MATERIALS AND METHODS**

**Sampling and Culture.** Samples were collected from eye, lung, lymph node of mammary glands, ear and milk secretion of goats from Qom province. Most of the samples were obtained from goat herds with clinical signs of a probable infection by \textit{Mycoplasma}. All the goats had been previously examined to confirm that they had clinical signs of C.A. Following this clinical examination, the samples (eye, ear, milk secretion and joint exudates) were taken and placed into the transport medium. Then the samples were transported to the \textit{Mycoplasma} reference laboratory of Razi Vaccine and Serum and Research Institute, Karaj, Iran.

**DNA extraction and PCR Sequence analysis of Mcc.** The specimens were diluted and filtered into the fresh PPLO broth and then inoculated on to PPLO agar medium (BBL, Becton Dickinson and company, Cockeysville, Sparks, MD, USA). Inoculated agar and broth were incubated at 37°C in 50% CO$_2$ and 98% humid atmosphere. The broths were observed daily for signs of growth and the plates were considered for the typical appearance of \textit{Mycoplasma} colonies. Mcc reference strain (NCTC 10154) has been used in this study as a positive control and uncultured PPLO broth as a negative. DNA was extracted from samples using a previously described method by Kojima et al. 1997 with some modifications. An initial PCR-based on detection assay for genus \textit{Mycoplasma} was performed.
(Kojima et al. 1997), then all the positive samples were analyzed by specific PCR procedure for Mycoides cluster (Bascunana et al. 1994). Finally all the positive samples which were positive in Mycoides cluster PCR were analyzed by another specific PCR procedure for diagnosing Mcc (Hotzel et al. 1994). The PCR assay was conducted in a Gradient Mastercycler (Eppendorff, Germany). Finally the visualization of the amplified products was done by UV illumination after electrophoresis (1% agarose gel in 1×Tris–acetic acid–EDTA (TAE) buffer) and ethidium bromide staining. They were purified from agarose gel using a High Pure PCR product purification kit (Roche Applied Science, Penzberg, Germany) according to the manufacturer’s instructions and sent to MWG Biotech Company (EurofinsMWG Operon, Ebersberg, Germany) for sequencing both isolates of Mcc were sequenced in both directions and then assembled, and edited using the DNAlib MAX 3.0 software (Hitachi, Pharmacia, Hitachi Software Engineering Company, Yokohama, Japan), the forward and reverse complemented sequences were compared to produce a consensus sequence using DNAlib and then were manually aligned by using the Bioedit Software version 7.1 (Hall 1999).

RESULTS
The 111 samples collected from goat herds which analyzed simultaneously by culture and Mycoplasma genus PCR (MPCR), M. cluster Mycoides PCR (M.M Cluster PCR) and finally Mcc PCR. 31 (27.9%) samples were positive and showed typical Mycoplasma colonies, and 80 (72.1%) samples scored negative for using culture method. 51 (45.9%) samples were scored positive for the presence of Mycoplasma and 58 samples (54.1%) were scored negative by PCR method. 8 (7%) samples were positive in Mycoplasma myciodes cluster PCR and 43(93%) samples were negative in Mycoplasma myciodes cluster PCR. 2 (1.8%) samples were scored positive in Mcc PCR (Figure 1). One of those samples, which were positive by Mcc PCR, detected from lung and the other one detected from lymph nodes (Table 1, 2). The conserved, surface protein 13S ribosomal protein S7 domain of rpsG gene was sequenced for both isolates. Then both strains which entitled MccR-2569 and MccR-2570 were compared by a reference strain that was reserved in Gene Bank Data base by ATC 27343 accession number. The results showed completely identical in that domain and the similarity of the microorganisms (Table 3).

DISCUSSION
Mcc is one of the etiological agents of C.A (Madanat et al. 2001). This pathogen in goats is highly destructive which causes high morbidity and mortality (DaMassa et al. 1992). PCR technique was developed 17 years ago for detection of Mycoplasmas which cause in C.A (Ameros et al. 2010, Hotzel et al. 1996). There are many investigations which confirm that PCR can be used as an alternative to culture method for detection of Mycoplasmas which involve in C.A (Abtin et al. 2013, Kheyrkhah et al. 2011, Ameros et al. 2010 and Johnson et al. 2004). The present study was based on an investigation in to existence of Mcc in goats of Iran. The results of this study showed that 27.9% of samples were positive by culture, 36.3% of the samples were infected by Mycoplasmas, 5.6% samples were belonged to myciodes cluster and 1.6% of the samples which were positive in myciodes cluster PCR, were Mcc. According to the results obtained, PCR were more successful in detection of Mycoplasmas than culture. On the other hand, both samples which were positive by MccPCR, were scored negative by culture. Therefore, the results of the present study were in agreement with other studies which were claimed PCR were more accurate than culture in detection of Mycoplasmas. In this study, it is observed that eye samples, milk samples, lymphoid node samples and lung samples were suitable samples for detecting of Mycoplasmas which belong to myciodes cluster. Also the results of this study demonstrated that Mcc was isolated from lymphoid liquid and lung samples. Awan et al. 2009 demonstrated that in 40% of the lung culture samples.
and in 40% of the nasal swabs, *Mcc* was detected.

### Table 1. Comparison of the results of culturing and PCR analysis for the detection of *Mycoplasmas* in samples.

<table>
<thead>
<tr>
<th>No. Sample</th>
<th>No. Culture</th>
<th>No. MPCR Cluster Mycoides PCR</th>
<th>No. Mcc PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>111</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>51</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>60</td>
<td>43</td>
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<tr>
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<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6</td>
</tr>
</tbody>
</table>

### Table 2. The culture and PCR methods results in different samples.

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Milk samples</th>
<th>Ear sample</th>
<th>Eye sample</th>
<th>Lung sample</th>
<th>Joint sample</th>
<th>Lymph nodes</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>Positive</td>
<td>6</td>
<td>2</td>
<td>17</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>22</td>
<td>26</td>
<td>29</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>8</td>
<td>7</td>
<td>27</td>
<td>2</td>
<td>6</td>
<td>1</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>20</td>
<td>21</td>
<td>19</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>Genus PCR</td>
<td>Positive</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>19</td>
<td>7</td>
<td>22</td>
<td>1</td>
<td>6</td>
<td>0</td>
<td>43</td>
</tr>
<tr>
<td>Cluster Mycoides PCR</td>
<td>Positive</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Mcc PCR</td>
<td>Negative</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
</tbody>
</table>

### Table 3. Results of Phylogenetic analysis of *Mcc*.

<table>
<thead>
<tr>
<th>Sequence-&gt;</th>
<th>CP000123.1</th>
<th>MccR-2569</th>
<th>MccR-2570</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP000123.1</td>
<td>ID</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>MccR-2569</td>
<td>100%</td>
<td>ID</td>
<td>100%</td>
</tr>
<tr>
<td>MccR-2570</td>
<td>100%</td>
<td>100%</td>
<td>ID</td>
</tr>
</tbody>
</table>
They claimed that the nasal swab and the lung samples of the goats are the best place for detecting of Mcc. The results of this study were in agreement with them for detecting of Mcc and isolation of Mcc from lung samples. In addition, the present study showed lymphoid node can be one of the good samples for attending of Mcc. De la Fe et al. 2007 detected 17.5% Mcc from goats and kids for the first time from Lanzarote Island in Spain. Results of this study confirmed detection of Mcc and prevalence of Mcc from goats of Lanzarote Island in Spain were higher than Qom province in Iran. Kumar et al. 2011 could detect 3% Mcc and 5% Mmc from goats of Gujarat state in India. Result of the present study were in agreement with Kumar et al regarding of detecting Mcc and prevalence of Mcc. yet in Kumar’s study, the population of goats were more than present study. The Results of the Kumar study showed that Mcc was highly detected from the nose samples of goat herds. Finally, the results of the present study showed lymphoid node and lung samples were significant samples for detecting of Mcc and it was in agreement with (Nicolass et al 2008, Bergonier et al 1997). While, Iran is one of the biggest countries in breeding of goats and sheep, it runs a significant risk of C.A which can cause huge economic loss. Therefore, vaccination has been developed and recommended for decreasing the rate of prevalence of the diseases in goat and sheep herds to prevent from economic loses. In conclusion, Mcc was detected for the first time in goats of Qom province in Iran. Therefore, Qom can be one of geographic distribution of Mcc in the Iran. In order to discover more accurate rates of Mcc in Iran, it is necessary to collect further causative samples from various provinces of Iran. Further investigation of the other Mycoplasma belonging to myciodes cluster is suggested, which were causative in C.A, to reveal the rate of actual incidence of them in this country. Currently, there is no investigation or reporting of C.A in Iran. Therefore, it is necessary to take preventative measures to control or eradicate the presence of CA in various provinces of Iran.

Ethics
I hereby declare all ethical standards have been respected in preparation of the article.

Conflict of Interest
Hereby, I declare "no conflict of interest exists" regarding submitted article.

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References


De la Fe, C., Gutierrez, A., Poveda, JB., Assuncao, P., Ramirez, AS. and Fabelo, F. (2007). First isolation  of Mycoplasma capricolum subsp capricolum one of the casual agents of caprine contagious agalactia on the island of Lanzarote (Spain). The Veterinary Journal 173: 440-442.


