INTRODUCTION

Contagious agalactia (C.A.) is a significant infectious disease in dairy sheep and goats. It is identified by mastitis, arthritis, pneumonia and keratoconjunctivitis (Nicolas 2008). It may be caused by any of the five Mycoplasma species associated with this disease, namely, M. agalactiae, Mycoplasma mycoides subsp mycoides LC (large colony), Mycoplasma capricolum subsp capricolum, Mycoplasma mycoides subsp Capri and Mycoplasma putrefaciens (Nicolas 2008).
Zendulkova et al 2007, Bergnioer et al 1997). However M. agalactiae is still regarded, particularly in sheep as the “classical” etiological agent of the C.A (Bergnioer et al 1997). M. agalactiae can be very contagious in goats and sheep. It appears to acute, sub acute or chronic disease (Greco et al 2001).

C.A has been reported in southern Europe (Bergonier et al 1997) and south of America and north of Africa and serious problem exist in Iran where over 1300 cases were reported in 2006 (OIE 2008). It often appears in a herd in the spring soon after lactation begins and probably represents the activation of latent infection and females transmitted infectious factor to lambs by milk, so the main target of C.A is mammary gland, where a fall in or complete loss of milk production sometimes within 2 or 3 days (Nicolas 2008). This infection occurred in the herds at any ages but the pregnant and lactating females are more susceptible (Aytu et al 1990). The latency of this disease is 1-2 weeks but they carry it out for 7 months after spending the period of the incubation (Aytu et al 1990). Since 1981 several Mycoplasma species have been identified in the external ear canal of goats, including all species involved in the etiology of CA syndrome (Amores et al 2010). The sign of keratoconjunctivitis is usually of short duration and it was seen in about 50% of infected animals and it may occasionally develop in to a chronic infection, occasionally resulting in unilateral or bilateral blindness (Mebuss 1998).

Culture can be costly and time-consuming, and can also be inconclusive (Kheirkhah et al 2011). Molecular diagnostic tests have been used to identify the M. agalactiae since last decade too, PCR assay have been one of those tests for detecting of M. agalactiae in milk samples directly (Tola et al 1997). M. agalactiae was isolated and identified by culture and PCR assays from goats and sheep of provinces of Iran (Kheirkhah et al 2011, Moradi Bidhendi et al 2011, Pirali et al 2007).

Because of the Qom province is located in central part of Iran and it is one of the most important provinces in terms of goats and sheep breeding. The incidence of C.A in this province has increased during these years. There is not any investigation for detecting the agent of this syndrome in that province so this leads to isolate and detect of M. agalactiae which is one of the main etiological agent of the C.A. The aim of the present study was designed to isolate and identify of M. agalactiae by culture and PCR assays from sheep of Qom provinces in Iran with clinical signs of the disease.

MATERIALS AND METHODS

Sampling and cultures. Samples were collected from eye, ear, milk secretion and joint exudates. Most of the samples were obtained from herds with clinical signs of a probably infection by Mycoplasma. All the sheep which were taken samples form them in this study 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 in to the transport medium then samples transported on ice to the Mycoplasma reference laboratory of Razi Vaccine and Serum and Research Institute, Karaj, Iran.

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 incubated at 37°C in 50% Co2 and 98% humid atmosphere. The broths were observed daily for signs of growth and the plates were considered for the typical appearance of Mycoplasma colonies. M. agalactiae reference strain (NCTC 10123) have used in this study as a positive control and uncultured PPLO broth as a negative control.

DNA extraction. DNA was extracted from samples using a previously described method by Pourbakhsh et al (2010) with some modifications. 0/5ml of each sample was transferred to Eppendorf tube and centrifuged for 15 min at 13000 rpm. The supernatant fluid was discarded and add lysis buffer (Tris-HCl 50 mM pH=8, SDS 1%, NaCl 100mM, EDTA 50 mM, proteinase K 20 µl to 200µl) to the tube equal volume
Table 1. The results of the culture and PCR methods in different samples.

<table>
<thead>
<tr>
<th>Tests</th>
<th>Result</th>
<th>Milk sample</th>
<th>Ear sample</th>
<th>Eye sample</th>
<th>joint sample</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>Positive</td>
<td>3(16.6%)</td>
<td>2(7%)</td>
<td>14(27%)</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>15(86.4%)</td>
<td>27(93%)</td>
<td>38(73%)</td>
<td>3(100%)</td>
<td>83</td>
</tr>
<tr>
<td>PCR</td>
<td>Positive</td>
<td>8(44%)</td>
<td>12(41.4%)</td>
<td>39(75%)</td>
<td>0</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>10(66%)</td>
<td>17(58.6%)</td>
<td>13(25%)</td>
<td>3(100%)</td>
<td>43</td>
</tr>
<tr>
<td>MAPCR</td>
<td>Negative</td>
<td>5(28%)</td>
<td>2(7%)</td>
<td>12(23%)</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>13(72%)</td>
<td>27(93%)</td>
<td>40(77%)</td>
<td>3(100%)</td>
<td>83</td>
</tr>
</tbody>
</table>
presence of *Mycoplasma* and 44 samples (43.2%) were scored negative by PCR method. 19(32.2%) samples were positive in *M. agalactiae* specific PCR, and 40(67.8%) were negative for using PCR method for *M. agalactiae*. *M. agalactiae* was detected in 19(32%) of the samples tested with both methods. 40 samples were MPCR positive-culture negative and there was only one sample that MPCR negative-culture positive, also 40 of the samples scored negative for using MPCR and MAPCR and no one of samples are MAPCR positive-MPCR negative (Table 1).

In this study after clinical examinations, samples were collected from the most significant lesions that were observed in each herd, eye samples were the most lesions that *Mycoplasma* was isolated and diagnosed by culture and PCR and joint exudates were the least lesions that *Mycoplasma* was isolated and diagnosed by culture and PCR, whereas milk samples were the most lesions that *M. agalactiae* was identified by MAPCR and joint exudates also were the least samples that *M. agalactiae* was detected by MAPCR (Table1). In PCR the DNA fragment of *Mycoplasma* genus with approximately 163 bp was amplified (Figure 1).

![Figure 1](image1)

The *M. agalactiae* PCR product was 375 bp in length (Figure 2).

![Figure 2](image2)

**DISCUSSION**

In this study, *M. agalactiae* was detected from mammary gland, conjunctive, ear and joint exudates samples of sheep herds of Qom province, Iran by using PCR and culture methods. PCR can be used more reliably on *Mycoplasma* growing in culture with 24 hour enrichment of the *Mycoplasma* in the appropriate medium greatly facilities PCR detection even in the presence of bacterial contamination (Nicolas 2008). PCR as a molecular detection method is routinely used in many laboratories and is extremely sensitive, it can provide a rapid early warning system when carried out on clinical samples, enabling a full investigation to take place when the results are positive, However the negative results should not be considered definitive and the detection limit for *M. agalactiae* in cultures has been brought to 2×10² CCU/ml (Dediu et al 1995, Tola et al 1997, Nicolas 2008, Bergonier 1997). The result of this study showed that PCR was more successful than the culture in detecting of *M. agalactiae* and was in agreement with the result of the recent reports Kheirkhah et al (2011) from Iran, Amores et al (2010) from Spain and Tola et al (1997) from Italy.
This study showed that, 23% of eye samples were positive in PCR with *M. agalactiae* primers, 7% of ear samples were positive in MAPCR, 28% of milk samples were positive in MAPCR and *M. agalactiae* was not detected from joint exudates in present study. Bidhendi et al (2011) showed that *M. agalactiae* was detected from milk samples of the healthful and suspected sheep herd of C.A from Kordestan province in Iran. In the present study, *M. agalactiae* was detected from milk samples of the healthful and suspected sheep herd of C.A so the results of this study were more reliable. Khierkhah et al (2011) have detected *M. agalactiae* from milk and joint exudates of goats; they have declared that the highest number of *Mycoplasma* colony was obtained from joint exudates. This study was in agreement with them to detect of *M. agalactiae* from milk samples, but it was contrary in detection of *M. agalactiae* from joint exudates. However, *M. agalactiae* was not detected from joint exudates. The isolation of *M. agalactiae* from joint exudates might be related to special maintenance condition of goats herd. It causes that joint exudates of sheep were not suitable for detection and identification of *M. agalactiae*. Amores et al (2010) have detected *M. agalactiae* from ear swab samples of goats. Therefore, this study was in agreement with that study in detection of *M. agalactiae* from ear samples.

Zendulkova et al (2007) claimed that the PCR method was not efficient for detecting of *M. agalactiae* from sheep and goats of Jordan, also the eye samples and ear samples were not suitable for isolation and identification of *M. agalactiae*. This study was contrary with them to show that the PCR could be efficient for detecting of *M. agalactiae*, as well as ear and eye samples were one of the best useful for diagnosing of *M. agalactiae*. Pirali et al (2007) have also detected *M. agalactiae* from eye and milk samples of sheep and goats. Therefore, this study was in agreement with detecting of *M. agalactiae* from eye and milk samples of sheep. This study was conducted on the suspected samples. It has more reliable result than the other studies such as Moradi Bidhendi et al (2011) that conducted on the samples with or without signs of C.A. Eventually the most and the least isolations of *M. agalactiae* were taken respectively from milk and joint exudates samples. The similar study in goats is required for phylogenetic analysis of *M. agalactiae* in field samples by applying the lipoprotein gene sequence in GeneBank to identify source of the infection.

In conclusion, *M. agalactiae* was detected for the first time from sheep of Qom province and the result of our finding confirmed that *M. agalactiae* was one of the main etiological agents of the C.A in this province. In order to complete this study, it is necessary to identify other species of *Mycoplasma* such as *Mycoplasma mycoides* or *Mycoplasma putrefaciens* or *Mycoplasma capricolum* in samples which were positive with genus using *Mycoplasma* PCR.

**Acknowledgements**

We thank all the staff of the Mycoplasma reference laboratory, Razi Vaccine and Serum Research Institute, Karaj, Iran. This study was supported by a project of Razi Institute and Education and Research Deputy of Jihad-Agriculture Ministry with the grant No. 2-18-18-89067.

**References**


