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Short communication

Occurrence of *Mycoplasma* in bovine mastitic milk in Iran

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Abstract

This study was conducted to determine the prevalence rate of Mycoplasma species in bovine raw milk samples from Iran using PCR assay. Based on CMT, out of 100 milk samples tested for mastitis, 62 and 80% were positive for Mycoplasma in cows and buffaloes respectively. Based on PCR results, the prevalence of mastitis was 48.67% and 51.33% in cows and buffaloes respectively.

Keywords: Mastitis, *Mycoplasma spp.*, PCR, Bovine, Milk

Introduction

Mycoplasma bovine mastitis is a highly contagious disease that results in milk loss and culling of infected animals. *Mycoplasma* spp. is the causative agent of several diseases in dairy cattle and calves, such as mastitis, pneumonia, arthritis, genital disorders and abortions (Gonzalez and Wilson, 2003; Ayling et al., 2004). Since the diseases are largely resistant to chemotherapy, it is necessary to have rapid and reliable diagnostic methods to detect the agents at an early stage. So that effective control measures can be introduced in time (Gonzalez et al., 1992; Byrne et al., 2005). However, current methods for the detection of *mycoplasma* are restricted to direct culture on mycoplasma agar media and serology, but both of these diagnostic methods are time consuming, laborious and difficult. Isolation and identification of Mycoplasma may take weeks and few laboratories have the capabilities required to culture Mycoplasma serodiagnosis, the demonstration of which is dependant upon antibody titres that is reached only ten to fourteen days after the onset of clinical symptoms (Ghadersohi et al., 1999; Nicholas and Ayling, 2003).

Consequently the pathogen cannot be detected during the incubation period. Moreover, the serological cross reactions among the Mycoplasma species are a critical problem (Pinnow et al., 2001; Bashiruddin et al., 2005). Therefore, molecular techniques such as PCR that is simpler, faster, less hazardous and usually more sensitive have been developed for Mycoplasma detection in clinical samples (Konigsson et al., 2002).

Recently, Houlihan et al. (2007) reported mastitis in dairy herds and recommended that veterinarians should consider Mycoplasma where there is unresponsive mastitis, particularly in view of the fact that at least 11% of cases of mastitis go undiagnosed.

Currently, there is limited information regarding the prevalence of mycoplasma species in raw milk in Iran. The present study was conducted to determine the prevalence of mycoplasma spp. isolated from bovine mastitic milk in shahrekord, Iran.

Materials and Methods

From March to May 2010, a total of 50 dairy cows were randomly selected from 10 commercial dairy farms of Chaharmahal va Bakhtiari, Iran. Also, 50 buffaloes were selected from 5 buffalo heard of Ahwaz, Iran. Then, 10 ml milk from each quarter (N=400) was collected into separate tubes. Collected sample were also examined using the Californian mastitis test (CMT).

DNA was extracted directly from milk samples by using DNA extraction and purification kit (Cinnagen, Tehran, Iran) according to manufacturer's instruction. Detection of *Mycoplasma* spp. was performed by amplification with the following primers: 5'-GCATGAAAGTAATATAAAAGGAAGCG-3'

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(forward) and 5'-GCTACTTTTTCTTCCCTAACCAC AG-3' (reverse), designed from the DNA sequence of 16S rRNA gene of Mycoplasma resulting in a 276 bp product.

The PCR assay was performed in a final volume of 25 µL mixture containing 50 mmol KCl, 10 mmol Tris-HCl (pH 8.3), 1.5 mmol $MgCl_2$, 0.2 mmol of each deoxynucleotide triphosphate, 0.5 µmol of each primer, 1.25 unit Taq polymerase (Cinnagen, Tehran, Iran) and 5µL of DNA template.

Reactions were initiated at 94°C for 5 min, followed by 30 cycles of 94°C for 1 min, 58°C for 1 min, 72°C for 2 min and a final elongation step at 72°C for 10 min, with a final hold at 4°C. Amplified samples were analyzed by electrophoresis (120 V/208 mA) in 1.5% agarose gel and stained by ethidium bromide.

Results and Discussion

As shown in Table 1, the incidence of subclinical mastitis, based on CMT was 62 and 80% in dairy cattle and buffaloes, respectively. These results indicate that subclinical mastitis was higher in buffaloes than dairy cattle in study areas. One reason for this might be the fact that buffaloes allowed to free grazing in infected area like dunghills.

Gel electrophoresis of amplicons confirmed that all primer pairs specifically amplified the desired PCR products. Each PCR product was obtained as a clear band at 276 bp, generated by Mycoplasma (Fig 1). In total, 226 of 400 (56.5%) milk samples were positive for presence of Mycoplasma DNA. Out of 226 positive samples 110 (48.67%) and 116 (51.33%) belonged to dairy cows and buffaloes, respectively.

Results of the present study are approximately similar to a recent report in Egypt that showed incidence of subclinical mastitis was 32.62 and 26.25% in cows and buffaloes, respectively. Also, examination of cows and buffaloes for Mycoplasma revealed 9.09 and 0% from subclinical mastitic animals and 14.73 and 14.29%, from clinically mastitic animals, respectively (Osman et al., 2008). In another study conducted in Ardabil state (Iran), out of 80 milk samples of dairy cows, 39 (48.75%) were positive for *M. bovis* (Ghazaei, 2006).

The results of this study indicate that Mycoplasma was detected as a causative agent of mastitis in the dairy cows in Iran. In addition, PCR assay could become a valuable diagnostic or screening test for detection of Mycoplasmal mastitis in dairy herds.

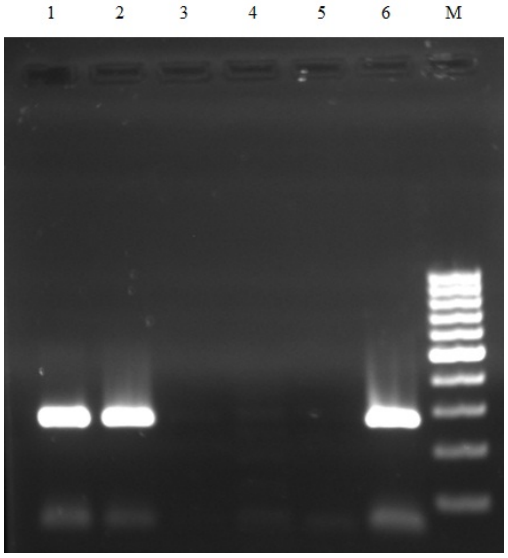

Fig. 1: PCR for detection of *Mycoplasma spp.* Lane 1 to 4, different samples from mastitis samples and lane 5 to 6 are negative and positive control, respectively.

Table 1: Prevalence of *Mycoplasma* from dairy cattle and buffalo milk samples (CMT based)

Species	No. herds studied	No. selection per herd	No. studied animals	Normal animals	Mastitic animals
Cow	10	4-7	50	19 (38%)	31 (62%)
Buffalo	5	10-13	50	10 (20%)	40 (80%)

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