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Original Article

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

Pourbakhsh*[1], S.A., Abtin[1], A.R., Ashtari[1], A., Bayatzadeh[1], M.A., Barani[2], S.M., Asli[1], E.

1. Mycoplasma Reference Laboratory, Razi vaccine and Serum Research Institute, Karaj, Iran
2. Veterinary General Office of Qom Province Veterinary, Qom, 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 *myciodes 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 putrefacients (M. putrefacients)* 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

*Author for correspondence.Email: a.pourbakhsh@rvsri.ac.ir

goats and cattle (Bergnioer *et al* 1997) as well. Tully et al. 1974 named *it 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).

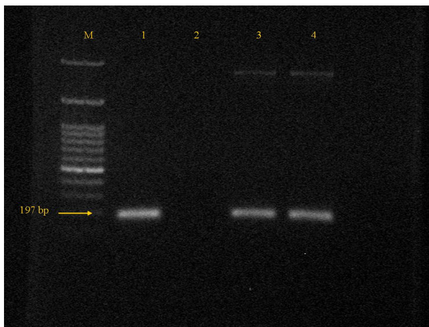

Figure1. *Mcc*PCR: PCR electrophoresis analysis in %1 gel agarose. M*:* Marker (100bp DNA ladder).*Lane 1*: Positive control (197bp band, *Mcc*, NCTC 10154).*Lane 2:* Negative control (uncultured PPLO broth) and *Lane 3 and 4* are the *Mycoplasma* isolates in this study.

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 *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 *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 *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, Cockeyville, 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 *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 *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 (EurofinsMWGOperon, Ebersberg, Germany) for sequencing both isolates of *Mcc* were sequenced in both directions and then assembled, and edited using the DNASIS 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 DNASIS 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 *Mcc*R-2569 and *Mcc*R-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 *Mcc*PCR, 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.

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

No. Sample	**No. Culture**		**No. *M*PCR**		**No. *Mycoplasma Cluster Mycoides* PCR**		**No. *Mcc* PCR**	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
111	**31**	**80**	**51**	**60**	**8**	**43**	**2**	**6**

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

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

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

Sequence->	**CP000123.1**	**MccR-2569**	**MccR-2570**
CP000123.1	ID	100%	100%
MccR-2569	100%	ID	100%
MccR-2570	100%	100%	ID

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 preventive 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

Abtin, AR., Pourbakhsh, SA., Ashtari, A., Bayatzadeh, MA., Barani, SM. and Ahangaran, S. (2013). Isolation and identification of *Mycoplasma agalactiae* by culture and polymerase chain reaction (PCR) from sheep of Qom province, Iran. *Archives of Razi Institute 68(1): 11-16.*

Amores, J., Corrales, JC., Martin, AG., Sanchez, A. and Conteras, A. (2010). Comparison of culture and PCR to detect *Mycoplasma agalactiae* and *Mycoplasma mycoides subsp. Capri* in ear swabs taken from goats. *Veterinary Microbiology* 140: 105-108.

Awan, MA., Abbass, F., Yasinzai, M., Nicholas, RAJ., Bbabar, S., Ayling, RD., Attique, MA. and Ahmed, Z. (2009). Prevalence of *Mycoplasma capricolum subsp capricolum* and *Mycoplasma Putrefaciens* in goats Pishin district of Balochistan. *Pakistan Veterinary Journal* 29(4): 179-185.

Bascunana, CR., Mattsson, JG., Bolske, G. and Johansson, KE. (1994). Characterization of the *16SrRNA* genes from *Mycoplasma* sp. Strain F38 and development of an identification system based on PCR. *Journal of Bacteriology* 176(9): 2577-2586.

Bergonier, D., Berthelot, X. and Pourmarat, F. (1997). Contagious agalactia of small ruminants current of knowledge concerning epidemiology diagnosis and control. *Review Science Techenology* 16(3): 848-873.

Corrales, JC., Esnal, A., De la Fe, C., Sanchez, A., Contreras, A., Sanchez, A., Assuncao, P., Poveda, JB. and Contreras, A. (2008). Contagious agalctia in small rumminats. *Small ruminant reasherch* 68: 154-166.

Cottew, GS., Breard, A., DaMassa, AJ, Emo, H., Leach, RH., Lefevere, PC., Rodwell, AW. and Smith, GR. (1987). Taxonomy of *Mycoplasma mycoides cluster*. *Isr, Journal. Medicine science* 23: 632-635.

Damassa, AJ., Brooks, DL., Holmberg, CA. and Moe, AI. (1987). Caprine Mycoplasmosis: an out break of mastitis requiring destruction of 700 goats. *Veterinary Record* 120(17): 409-413.

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.

Dedieu, L., Mady, V. and Lefevre, PC. (1995). Development of two PCR assay for the identification of *Mycoplasma* cauasing contagious agalactia. *FEMS Microbiology Letters* 129: 243-250.

Foggie, AA., Etheridge, JR., Erdag, O. and Arisoy F. (1971a). Contagious agalactia of sheep and goats studies on live and dead vaccines in lactating sheep. *Journal. Comp. Pathol* 81: 165-172.

Foggie, AA., Etheridge, JR., Erdag, O. and Arisoy, F. (1971b). Contagious agalactia of sheep and goats studies on live and dead vaccines in lactating sheep. *Journal. Comp. Pathol* 81: 393-400.

Hall, T.A. (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series,* 41: 95-98.

Hasso, S., AL-Aubaidi, JM. and AL-Darraji ,AM. (1993). Contagious agalactia in goats: It is severity related to the route of infection ands pregnancy. *Small ruminants reviw* 10: 263-275.

Hotzel, H., Sachse, H. and Pfutzner, H. (1996). A PCR scheme for differentiation of organisms belonging to the *Mycoplasma mycoides cluster*. *Veterinary Microbiology* 49: 31-43.

Johnson, LR., Drazenovich, NL. and Foley, JE. (2004). A comparison of routine culture with polymerase chain reaction technology for the detection of *Mycoplasma* species in feline nasal samples. *Veterinary Diagnose Invest* 16: 347-351.

Kheirkhah, B., Pourbakhsh, SA., Nadalian, MG., Banani, M. and Ashtari, A. (2011). Detection of *Mycoplasma agalactiae* by culture and polymerase chain reaction (PCR) methods from Iranian goats. *African Journal of Microbiology Research* 5(13): 1668-1672.

Kojima, A., Takahashi, T., Kijima, M., Ogikubo, Y., Nishimura, M., Nishimura, S., Arasawa R. and Tamura, Y.(1997). Detection of *Mycoplasma* in avian live virus vaccines by polymerase chain reaction. *Biologicals* 25: 365-371.

Kumar, P., Roy, A., Bhanderi, BB. and Pal, BC. (2011). Isolation and molecular characterization of *Mycoplasma* isolates from goats of Gujarat State, India. *Veterinarski Archive* 81(4): 443-458.

Lambert, M. (1987). Contagious agalactia of sheep and goats. *Review science technology office int epiz* 6(3): 699-711.

Madanat, A., Zendulkova, D. and Pospisil, Z. (2001). Contagious agalactia of sheep and goats. *ACTA Veterinary Brno* 70: 403-412.

Monnerat, MP., Thiaucourt, F., Nicolet, J. and Frey, J. (1999). Comparative analysis of the *lppA* locus in *Mycoplasma capricolum subsp capricolum* and *Mycoplasma capricolum subsp caprineumoniae. Veterinary Microbiology* 69(1999): 157-172.

Moradi Bidhendi, S., Khaki, P. and Pilechian Langroudi, R. (2011). Isolation and identification of *Mycoplasma agalactiae* by culture and Polymerase Chain Reaction in sheep and goat milk samples in Kordestan province, Iran. *Archives of Razi Institute* 66: 11-16.

Nicholas, R., Ayling, R. and McAuliffe, L. (2008). *Mycoplasma* Diseases of ruminants. *Small Ruminant Research* 98-114.

OIE. (2008). Contagious agalactia. Chapter 2.7.5.

Tully, JG., Barile, MF., Edward, DG., Theodores, TS. and Erno H. (1974). Characterization of some caprine *Mycoplasma* with proposals for new species, *Mycoplasma capricolum capricolum* and *Mycoplasma Putrefaciens*. *Journal. Genetic. Microbiology* 85: 102-120.

Woubit, S., Manso-Silvan, L., Lorenzon, S., Gaurivaud, P., Poumarat, F., Pellet M-P., Singh, VP. and Thiaucourt, F. (2007). A PCR for detection of *Mycoplasmas* belonging to the *Mycoplasma mycoides cluster*: Application to the diagnosis of Contagious agalactia. *Molecular and cellular probe* 21: 391-399.