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Identification of *Mycoplasma capricolum* subsp. *capricolum* causing MAKePS syndrome in small ruminants by PCR

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Mycoplasma capricolum subsp. *capricolum* (Mcc) is a pathogen of small ruminants associated with caprine pneumonia, polyarthritis, mastitis, septicaemia ovine arthritis and vulvovaginitis in different parts of the world. It belongs to the capricolum group within the *Mycoplasma mycoides* cluster- a group of six organisms with remarkably similar biological, serological and genetic features.

Moreover, Mcc shares extensive serological, antigenic and genetic homologies with other members of *M. mycoides* cluster especially *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp), the etiological agent of CCPP and *Mycoplasma* species bovine group 7 (Bonnet *et al.* 1993) impeding the proper identification and diagnosis of infection caused by Mcc. The advent of DNA based technologies like PCR and southern hybridisation and their application in diagnostic mycoplasmology have resulted in improved identification and differential detection of Mcc strains (Dedieu *et al.* 1992, 1994, Hotzel *et al.* 1996, Monnerat *et al.* 1999). The present study was undertaken with the objective of specific detection and differentiation of *Mycoplasma capricolum* subsp *capricolum* from other common ruminant mycoplasmas by PCR.

The strains used in the study were obtained from the National Referral Laboratory on Mycoplasma, Division of Bacteriology and Mycology, Indian Veterinary Research Institute, Izatnagar (Table 1).

All the strains were grown in MBHS-L medium (Singh 1990) except *Mycoplasma capricolum* subsp. *capripneumoniae* (F-38) and *Mycoplasma* species bovine group 7 (PG 50) which were grown in Hayflick's medium (Freundt and ErnØ 1979) and incubated at 37°C for 48-96 hr. The strains were characterized by digitonin sensitivity, biochemical and growth inhibition tests (Srivastava 1982).

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Table 1.*Mycoplasma* strains used in the study

Species	Strain
M. capricolum subsp. *capricolum*	California kid (Type strain)
	BMET
M. mycoides subsp. mycoides SC	*PG 1 (Type strain)*
	NCVP 1/86
M. mycoides subsp. *mycoides* LC	Y- Goat (Type strain)
	NCVP 2/99
M. mycoides subsp. *capri*	PG 3 (Type strain)
	VPNC 135
	VPNC 137
	Bhuj
M. capricolum subsp. *capripneumoniae*	F-38 (Type strain)
M. species bovine group 7	PG 50 (Type strain)
M. bovis	NC 317
	23 K
M. agalactiae	RPNS 200
	RPNS 216
A. laidlawii	NC 313
M. bovirhinis	NC 448
M. bovigenitalium	NC 58
M. alkalescens	NC 242

Genomic DNA from each mycoplasma was extracted as per Wilson (1987).Culture lysates for direct PCR were prepared from 1 ml of outgrown suspension following centrifugation at 12 500 rpm for 10 min and subsequent washing in 0.5 ml of PBS. The washed cells were resuspended in 50 µl of sterile distilled water, lysed by boiling for 20 min and stored immediately at –20°C till further use.

PCR was carried out to differentiate the *M. mycoides* cluster member from some of the other common ruminant mycoplasmas and to differentiate Mcc from other closely related cluster members. All reactions were carried out in a thermocycler. The reaction mixture was made up of 10x PCR buffer (100mM Trisma HCl pH 8.3 at 25°C, 500 mM KCl, 15 mM $MgCl_2$ and 0.001% w/v gelatin). 10mM dNTP mix, 25 p mol of each forward and reverse primer, 1 unit of Taq

DNA polymerase approximately 100 ng of template DNA or 2.5 µl of culture lysate and distilled water to make up 25 µl.

The *M. mycoides* cluster specific primers P1 (5'-TAT ATG GAG TAA AAA GAC – 3') and P2 (5' – AAT GCA TCA TAA ATA ATT G – 3') (Hotzel *et al.* 1996) were used at cycling conditions of 94°C for 30 sec, 46°C for 1 min and 72°C for 1.5 min for 30 cycles coupled with an initial 2 min denaturation at 94°C and final extension of 5 min at 72°C. The Mcc specific PCR used the primers P8 (5' – GTA AAC CGT GTA TAT CAA AT–3') (Hotzel *et al.* 1996) and MC 358 (5'–GAT ATC TAA AGG TGA TGG T – 3') (Bashiruddin *et al.* 1994) in 30 cycles of 94°C for 30 sec, 60°C for 45 sec and 72°C for 1.5 min with an initial denaturation and final extension as with P1/P2.

Following PCR, a 5 µl aliquot of each PCR product was subjected to electrophoresis on 1% agarose gel containing 0.5 µg/ml ethidium bromide in 1x TAE buffer along with pBR 322 *Hinf* I digest at 5v/cm for 2.5 hr. The gels were exposed and photographed under uv gel documentation system.

The study was carried out for the rapid and specific detection of Mcc strains and their differentiation from other members of mycoides clusters, and other common ruminant mycoplasmas. For this 2 sets of PCR assays were employed, one detecting all the *M. mycoides* cluster member together and the other one specific for Mcc strains alone. Amplification using *M. mycoides* cluster specific primer pair of P1/P2 yielded approximately 260 bp long amplicons with all the strains of the 6 cluster members (Fig 1). The non cluster mycoplasmas like *M. agalactiae, M. bovis, M. bovirhinis, M. bovigenitalium, M. alkalescens* and *A. laidlawii* failed to yield any amplified product. Similar results were observed by Hotzel *et al.* (1996), and this further confirms the utility of this test for differentiation of cluster and non cluster mycoplasmas. A positive result in this PCR will thus rule out the involvement of *M. agalactiae* from suspected cases of contagious agalactia or MAKePS syndrome caused by Mcc in small ruminants. The ability of this test to differentiate *M. mycoides* cluster organisms from other mycoplasmas implicated in similar

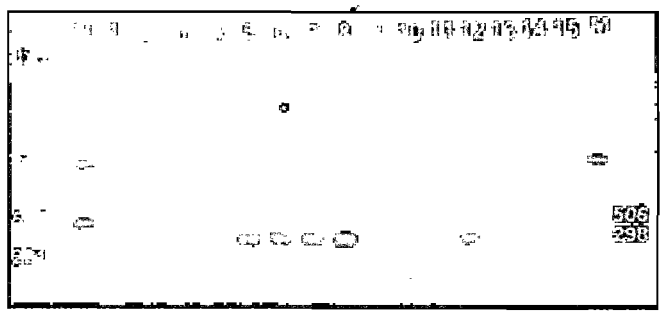

Fig. 1. PCR carried out using *Mycoplasma mycoides* cluster specific primers P1/P2. Lane. M- pBR 322/ *Hinf* I digest, 1- PG 1, 2- NCVP 1/86, 3- Y-Goat, 4- NCVP 2/99, 5-PG 3, 6- Bhuj, 7-VPNC 135, 8- VPNC 137, 9- California kid, 10-BMET, 11- F 38, 12-PG 50, 13- RPNS 200, 14- NC 317, 15- NC 313.

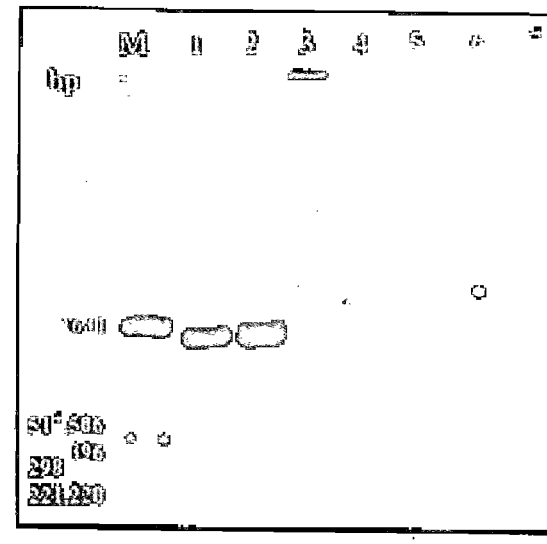

Fig. 2. PCR carried out using *Mycoplasma capricolum* subsp. *capricolum* specific primers P8/MC 358. Lane. M-pBR 322/*Hinf* I digest, 1-California kid, 2-BMET, 3-F 38, 4-PG 50, 5-PG 1, 6-Y-Goat, 7-PG 3.

clinical syndromes like *M. putrefaciens* could not be evaluated due to the non availability of strains. Moreover, there seems to be no report of isolation of *M. putrefaciens* from India.

Further differentiation of Mcc strains from other caprine and ovine mycoplsmas of the cluster could be accomplished by using the primers P8/MC358. PCR using this primer combination yielded approximately 1.4 kbp product from only the 2 Mcc strains (Fig. 2). Thus, this PCR could differentiate Mcc from other mycoplasmas causing MAKePS syndrome like Mmm LC and Mmc and also from the more genetically and antigenically related Mccp and Mbgr7. The direct application of this PCR assay on culture lysate without prior isolation of genomic DNA has got the added advantage of arriving at a rapid diagnosis, in a matter of a few hours, compared to the lengthy and cumbersome proccdures involved in serological and biochemical methods.

SUMMARY

The present study evaluates the applicability of PCR for specific detection and differentiation of *Mycoplasma capricolum* subsp. *capricolum* (Mcc) from other mycoplasmas involved in the pathogenicity of MAKePS syndrome in sheep and goats. The specific positive amplification signals observed with *M. mycoides* cluster and Mcc specific primers confirms the suitability of PCR for detection of Mcc strains.

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