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First report on the molecular prevalence of *Mycoplasma capricolum* subspecies *capripneumoniae* (*Mccp*) in goats the cause of contagious caprine pleuropneumonia (CCPP) in Balochistan province of Pakistan

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Abstract Contagious caprine pleuropneumonia (CCPP) caused by *Mycoplasma capricolum* subspecies *capripneumoniae* (*Mccp*) is a disease of goats which causes high morbidity and mortality and is reported in many countries of the world. There are probably no reports on the molecular prevalence of *Mccp*, *Mycoplasma capricolum* subsp. *capricolum* (*Mcc*) and *Mycoplasma putrefaciens* (*Mp*) in Balochistan and any other part of Pakistan. Thirty goats ($n = 30$) with marked respiratory symptoms were selected and procured from forty goat flocks in Pishin district of Balochistan in 2008. The genomic deoxyribonucleic acid (DNA) from the lung samples ($n = 30$) of the slaughtered goats was purified and subjected to polymerase chain reaction (PCR) assays for the presence of *Mycoplasma mycoides* cluster members and *Mp*. The PCR-RFLP (restriction fragment length polymorphism) was also used to further confirm the *Mccp*. Of the thirty lung samples 17 (56.67%) were positive for the molecular prevalence of *Mcc*, *Mccp* and *Mp*. In total the molecular prevalence was observed as 17.65% for *Mccp* ($n = 3$), 70.59% for *Mcc* ($n = 12$) and 11.76% for *Mp* ($n = 2$). The RFLP profile has also validated the PCR results of *Mccp* by yielding two bands of 190 and 126 bp. The results of PCR-RFLP coupled with the presence of fibrinous pleuropneumonia and pleurisy during postmortem of goats ($n = 3$) strongly indicated the prevalence of CCPP in this part of world. Moreover the prevalence of *Mcc* and *Mp* is also alarming in the study area. We report for the very first time the molecular prevalence of *Mcc*, *Mccp*, *and Mp* in the lung tissues of goats in the Pishin district of Balochistan, Pakistan.

Keywords Goats · Mycoplasma · CCPP · PCR · Balochistan · Pakistan

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Introduction

Mycoplasma organisms belong to the class Mollicutes of bacteria which have no cell wall and are notable for inflicting number of infections in animals [1] and humans [2]. Contagious caprine pleuropneumonia (CCPP) is an Office International Epizootes listed disease. It is a severe disease of goats caused by *Mycoplasma capricolum* subspecies *capripneumoniae* (*Mccp*) and is closely related to three other mycoplasmas: *Mycoplasma mycoides* subspecies *mycoides* Large Colony (*Mmm*LC), *Mycoplasma mycoides* subspecies *capri* (*Mmc*), and *Mycoplasma capricolum* subspecies *capricolum* (*Mcc*) [3]. Recently, *Mycoplasma mycoides* subspecies *capri* (*Mmc*) has been reported as a new designation for both of the *Mmm*LC and *Mmc* organisms [4].

Contagious caprine pleuropneumonia is usually observed with higher rates of morbidity and mortality when compared to pneumonia caused by other *Mycoplasma* species [5]. The isolation of *Mccp* requires a high level of expertise, a very special growth medium as this organism is very fastidious and at least 5–7 days initial incubation at 37°C with up to 5% carbon dioxide. The identification based on classical biochemical and growth inhibition (GI) tests is still

not definitive as these organisms are antigenically closely related. The use of PCR in detecting *Mycoplasma mycoides* cluster members, sub cluster members [6], and *Mccp* [7] is highly useful in diagnostic mycoplasmology. The specific PCR for *Mccp* is based on arcD gene in which a specific amplification of a 316 bp long DNA fragment is carried out which effectively rules out any confusion with other closely related members of *M. mycoides* cluster. Moreover, the application of Restriction Fragment Length Polymorphism on PCR products of *M. mycoides* subcluster members [6] and *Mccp* [7] further confirm the results of the respective PCR assays besides gene sequencing.

Balochistan is the southwestern province of Pakistan, on the eastern part of the Iranian plateau between 250 to 320 N latitude and 600 to 720 E longitude and is the largest of the four provinces in the country [8]. Of the 53.8 million goats in Pakistan many are produced on small farms which are distributed almost evenly across the country. The production systems are nomadic, transhumant and sedentary. The 11.8 million goats in Balochistan [9] are prone to many diseases because of the very cold winters in many areas of Balochistan and poor animal husbandry practices. There are reports of CCPP with high morbidity and mortality rates in many parts of Balochistan [10]. The use of a killed saponin adjuvated vaccine prepared from *Mmc* (Vaccinal strain) is still not that promising due to the prevalence of other *Mycoplasma* species having heterogeneous antigens. Pishin is one of the larger districts in Balochistan with temperatures ranging from −14°C in winter to 40°C in summer. There is continuous migration of small ruminants from Afghanistan to adjoining parts of Balochistan with no or partial enforcement of quarantine regulations. These small ruminants are mostly dependent on the natural vegetation for their survival.

Previous work on *Mycoplasma* diseases in goats in Balochistan, Pakistan has been limited [10, 11] and no research work on molecular level has yet been reported in Balochistan. This report describes for the first time the molecular prevalence of *Mccp*, *Mcc* and *Mp* organisms by using PCR assays on the lung samples of goats from the Pishin district of Balochistan.

Materials and methods

Forty goat herds with a total population of 2010 goats were visited during 2008 in the Pishin district of Balochistan. The goats were grossly examined for the presence of respiratory symptoms suspected for CCPP or caprine pneumonia. Over all thirty goats were selected and purchased from various herds and transported to the Center for Advanced Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan, Quetta.

The goats ($n = 30$) were slaughtered and post-mortem examination was performed for each of the goats. Samples from the lungs of each goat were collected and stored at −20°C until used for testing. The DNA was purified from each of the goat lung samples ($n = 30$) using the genomic DNA purification kit (PUREGENE–Gentra System, USA). The purified genomic DNA samples were stored in micro tubes (1.5 ml) at −20°C until used in specific PCR for the *Mycoplasma* species.

Polymerase chain reactions (PCR) for the detection of *M* "*mycoides* cluster" members, *M mycoides* sub cluster (*Mmm*SC, *Mmc*, *Mmm*LC) members [6], *Mccp* [7], and *Mp* [12] were performed on DNA samples (1 µl) purified from lung samples of goats in total reaction volume of 50 µl in Thermal cycler (Model # 2720, Applied Biosystem). All the primers used in this study were synthesized from Gene-Link USA. The PCR products (10 µl) were electrophoresed (100 V for 35 min) in mini gel unit (Elite-300, Wealtec, USA) by using 2% agarose (Vivantis, USA) and finally viewed in gel documentation system (Dolphin view-Wealtec, USA).

The primers used for PCR detection of *M* "*mycoides* cluster" members were MC323: 5′-TAG AGG TAC TTT AGA TAC TCA AGG 3′ (Forward) and MC358: 5′-GAT ATC TAA AGG TGA TGG T 3′ (Reverse). The primers used for *M* "*mycoides* sub cluster" members (*Mmm*SC, *Mmc*, *Mmm*LC) were MM450: 5′-GTA TTT TCC TTT CTA ATT TG 3′ (Forward) and MM451: 5′-AAA TCA AAT TAA TAA GTT TG 3′ (Reverse). Briefly the PCR cycling conditions for *M* "*mycoides* cluster" members and *M* "*mycoides* sub-cluster" members were 94°C for 4 min, followed by 33 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 30 s, followed by 72°C for 7 min. The amplicon sizes of 1500 bp and 574 bp were produced by *M* "*mycoides* cluster" and *M* "*mycoides* sub cluster" members, respectively.

The primers used for PCR detection of *Mccp* were Mccp-spe-F: 5′-ATC ATT TTT AAT CCC TTC AAG-3′ and Mccp-spe-R: 5′-TAC TAT GAG TAA TTA TAA TAT ATG CAA-3′. The PCR reaction conditions of 94°C for 2 min, followed by 35 cycles of 30 s at 94°C, 15 s at 47°C, 15 s at 72°C, 5 min at 72°C. *Mycoplasma capricolum* subspecies *capripneumoniae* produces an amplicon size of 316 bp. The PCR results of *Mccp* were further confirmed by subjecting the PCR product (5 µl) to RFLP using 2 µl of *Vsp1* restriction endonuclease (Vivantis, USA) in reaction mixture containing 2 µl and 1 µl of PCR grade water and 10× enzyme buffer (Vivantis, USA), respectively. The reaction mixture (10 µl) contained in PCR tube (0.2 µl) is electrophoresed (100 V for 35 min) in 3% agarose gel prior to 30 min incubation at 37°C in water bath.

The primers used for the detection of *M. putrefaciens* by PCR were SSF1: 5′-GCG GCA TGC CTA ATA CAT

GC-3′ and SSR1: 5′-AGC TGC GGC GCT GAG TTC A-3′. The PCR reaction conditions were 94°C for 5 min, followed by 25 cycles of 94°C for 30 s, 64°C for 30 s, and 72°C for 30 s, followed by 72°C for 7 min. *Mycoplasma putrefaciens* produces an amplicon size of 800 bp.

As there is no specific PCR available for the detection of *Mcc*, DNA samples were initially confirmed as positive for the presence of *M* "*mycoides* cluster" members [6], and negative for *M* "*mycoides* sub cluster" members [6] and *Mccp* [7] PCR assays. Of the remaining two *Mycoplasma* species the bovine group 7 type of *Mycoplasma* is so far not prevalent and reported in goats, therefore these DNA samples were considered as positive for *Mcc* organisms.

Results

Post mortem examination of the investigated goats showed pneumonia ($n = 27$) of various degrees in the slaughtered goats. Just three of these goats had serous fluid accumulating in the pleural cavity.

The results of PCR based molecular prevalence of *Mccp* ($n = 3$), *Mcc* ($n = 12$), and *Mp* ($n = 2$) in DNA purified directly from the lung samples of goats suspected for CCPP and pneumonia are shown in Table 1. In addition, DNA purified from the pleural fluid ($n = 3$) samples of the goats (The lung samples of these goats were already positive for the presence of *Mccp* through PCR) was also positive for *Mccp*. The PCR profiles for *M. mycoides* cluster members are shown (Fig. 1). Significant differences between the levels of positive amplicon were also observed. None of the DNA samples was positive for the presence of *Mmm*LC, *Mmc* and *Mmm*SC during specific PCR for *M. mycoides* sub cluster members (Fig. 2). Moreover the PCR profiles for the presence of *Mp* in lungs of goats are shown (Fig. 3).

Restriction fragment length polymorphism (RFLP) on the 316 bp PCR product gave a profile with bands of 190 and 126 bps which confirmed the presence of *Mccp*, the cause of CCPP, in the three goats suspected of having CCPP (Fig. 4).

Table 1 Prevalence of *Mycoplasma species* in goat lungs samples as detected by PCR

Number	*Mycoplasma* species	Sample +ve in PCR	Percentage
1	*Mcc*	12	70.59
2	*Mccp*	03	17.65
3	*Mp*	02	11.76

Mcc, *Mycoplasma capricolum* subsp. *capricolum*; *Mccp*, *Mycoplasma capricolum* subspecies *capripneumoniae*; *Mp*, *Mycoplasma putrefaciens*

Finally the DNA purified from lung samples of goats ($n = 12$) which were positive for specific PCR for *M* "*mycoides* cluster" members, negative for *M* "*mycoides* sub cluster" members and *Mccp* PCR assays were considered as positive for the presence of *Mcc* organisms.

Discussion

Caprine pleuropneumonia and other clinical manifestations due to *Mycoplasma species* are common in Balochistan. Despite the extensive use of *Mmc* based bacterin and antibacterial therapy, there are still reports of suspect CCPP, pneumonia, pleurisy, arthritis, kerato-conjunctivitis with high morbidity and mortality rates in the study area and other parts of this province.

The isolation and identification of *Mycoplasma* requires specific expertise in media preparation, collection and processing of samples, purification and characterization of *Mycoplasma* isolates based on classical biochemical, serological and molecular tests. Isolation of the fastidious *Mycoplasma* species such as *Mccp* is considered as one of the most difficult tasks and requires a special laboratory facility. In this study the molecular prevalence of *Mccp*, *Mcc* and *Mp* was undertaken by using PCR assays for the very first time in Pishin district of Balochistan, Pakistan. Previously there have been few reports on the isolation of *Mmc* from the lung tissues of goats on the basis of biochemical and serological techniques in some of the areas of Balochistan. [10, 11].

The PCR may be used as a diagnostic test both qualitatively and quantitatively. In the present study all the PCR tests used were qualitative. The presence of a specific amplicon is indicative of the antigen/disease being present in an animal. In some cases the specificity of the PCR is confirmed by RFLP. The PCR is not quantitative as the concentration of the target/organism DNA can not be standardized in the clinical sample. Hence the PCR amplicon concentration does vary between samples as shown in Fig. 1, where lane 9 shows a weak but positive amplicon. Furthermore; the difference in levels of amplicon produced is purely due to the variations of target organism present in the clinical sample/lung. The PCR's used in the present study are also used widely within the Mycoplasma community in most of the countries and internal controls are not commonly used by anyone else as the PCR's in effect just give a positive or negative result [6, 7, 12–14].

Undoubtedly, previous studies have demonstrated that *Mycoplasma* infections in goats are widespread within Pakistan particularly in Balochistan [10]. We have now introduced molecular methods into our laboratory to detect and identify these organisms that cause such economically

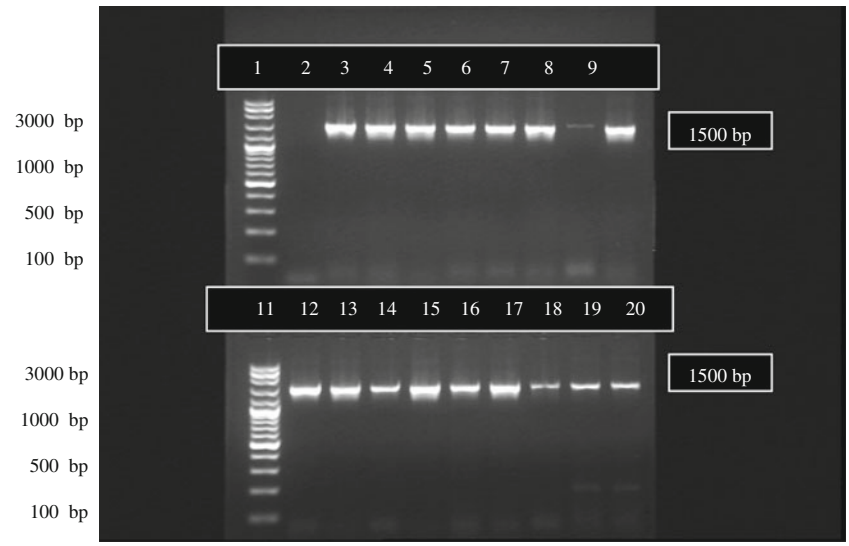

Fig. 1 Use of PCR to detect *M. mycoides* cluster members in the lung samples of goats showing an amplicon size of 1500 bp. Lane 1 & 11: Molecular Ladder (100 bp increment); Lane 2: –ve control; Lanes 10 & 20: *Mmm*LC and *Mccp standard* +ve controls, respectively; Lanes 3, 4, 5, 6, 7, 8, 9, 12, 13, 14, 15, 16, 17, 18 and 19: lung samples which are PCR positive for *M. mycoides* cluster members. (The PCR results are qualitative, and performed with proper positive and negative controls, which indicate that the PCR product is specific, therefore; internal controls are not included)

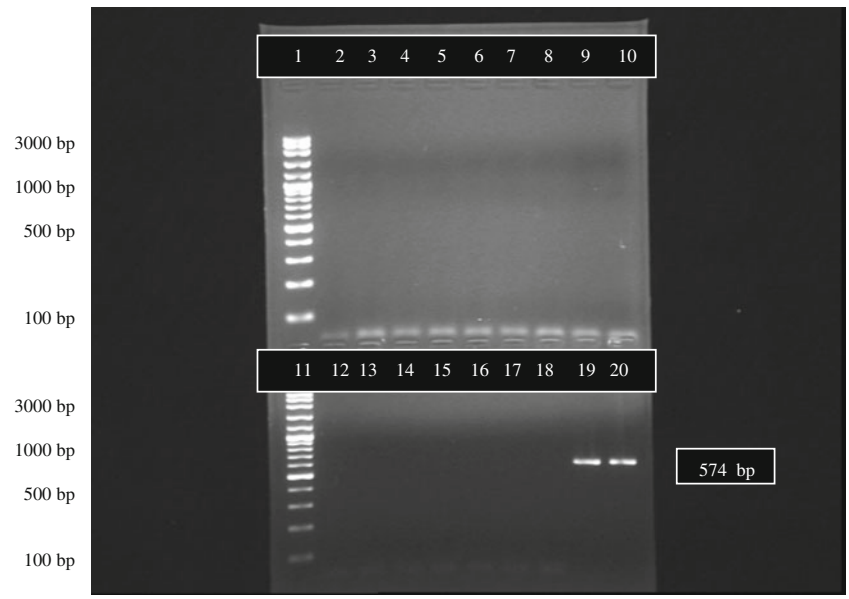

Fig. 2 Use of PCR to detect *M. mycoides* sub cluster members in the lung samples of goats showing an amplicon size of 574 bp. Lane 1 & 11: Molecular Ladder; Lane 2: –ve control; Lanes 19 & 20: *Mmc and Mmm*LC standard +ve controls, respectively; Lanes 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17 and 18: lung samples which are negative for *M. mycoides* sub cluster members. (The PCR results are qualitative, and performed with proper positive and negative controls, which indicate that the PCR product is specific, therefore; internal controls are not included)

important diseases. However traditional tests methods are still required to confirm the identification of some *Mycoplasma* isolates, specifically *Mcc*. There is therefore a need to develop more specific PCR test for *Mcc* to improve molecular diagnosis to enable the quick implementation of treatment and control measures.

In this study the molecular prevalence of *Mcc* and *Mp* in the lungs and *Mccp* in both the lung and pleural fluid samples from goats suspected of having CCPP and caprine pneumonia is quite alarming and of special interest as this is the first report on the presence of CCPP in Pakistan. Moreover, the results of PCR-RFLP for *Mccp* by using *Vsp1* enzyme are similar to the results [7] in which the same restriction endonuclease has cleaved the 316 bp PCR product of *Mccp* into two bands of 190 and 126 bp, thus confirming the diagnosis of CCPP.

Mycoplasma capricolum subsp. *capricolum* is highly invasive and may cause outbreaks similar to contagious agalactia (CA) and septicaemia disease seen with the *Mmc*. Fatal septicaemic polyarthritis may occur in kids fed with contaminated milk with *Mcc* whereas sporadic arthritis and abscesses may be less serious manifestations of *Mcc* [15]. The prevalence of *Mcc* in this study is also in agreement with the results of [13] in which 11 isolates of *Mcc* were detected from milk and nasal cultures of goats in Jordan, using PCR assays.

Mycoplasma putrefaciens is also one of the causative agents of CA syndrome [4]. Besides mastitis it is also

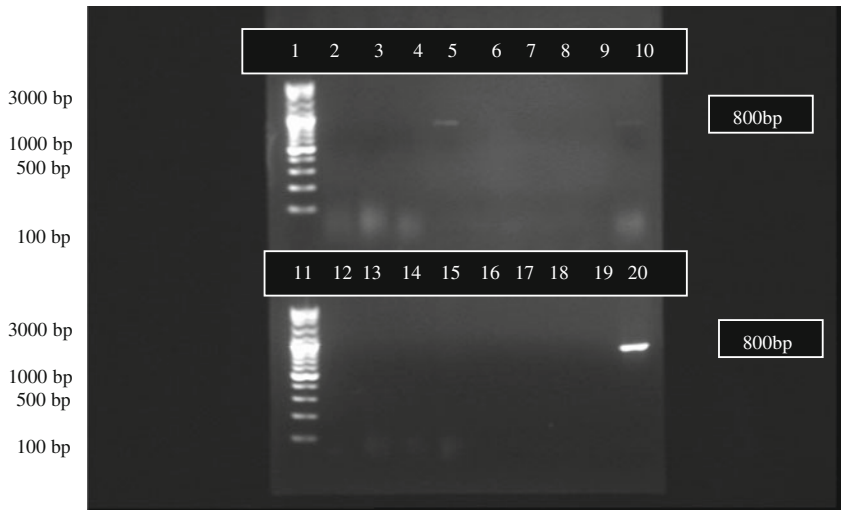

Fig. 3 Use of Specific PCR to detect *Mycoplasma putrefaciens* (*Mp*) in the lung samples of goats showing an amplicon size of 800 bp. Lanes 1 and 11: Molecular Ladder; Lane 2: –ve control; Lane 20: *Mp* standard +ve control; Lanes 5 and 10: Lung samples that are positive for *Mp* (lane 10 showing faint band); all other lanes having lung samples are negative for *M. putrefaciens.* (The PCR results are qualitative, and performed with proper positive and negative controls, which indicate that the PCR product is specific, therefore; internal controls are not included)

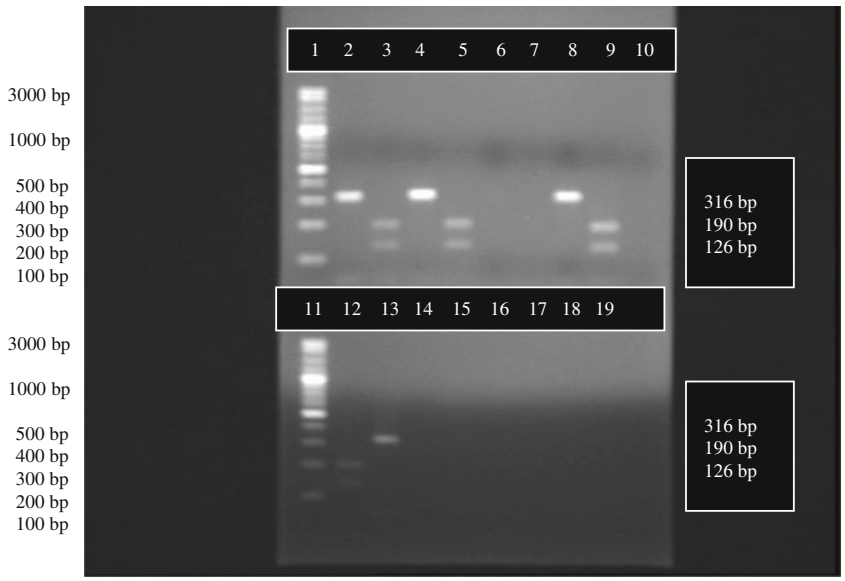

Fig. 4 Use of Specific PCR to detect *Mycoplasma capricolum* subsp. *capripneumoniae* (*Mccp*) in the lung samples of goats showing an amplicon size of 316 bp. Lane 1 & 11: Molecular ladder; Lane 2: PCR product of standard positive control *Mccp*; Lane 3: RFLP for standard positive control Mccp. PCR product (316 bp) cleaved with *Vsp1* into 190 and 126 bps bands; Lanes 4, 8 and 13: PCR product of the three lung samples positive for *Mccp*; Lanes 5, 9 and 12: +ve RFLP results for PCR product of DNA samples from lungs of the three CCPP goats (RFLP profile is faint in lane 12); Lanes 14–20: unused wells; (The PCR results are qualitative, and performed with proper positive and negative controls, which indicate that the PCR product is specific, therefore; internal controls are not included)

reported to cause septicaemia in kids and arthritis in adults [14]. It was also reported that *Mp* can be isolated from animals with and without clinical signs [16] suggesting a carrier status could occur. In this study, the PCR based detection of *Mp* in the lung samples can not be ignored and extensive studies are required to explore its isolation and characterization in this part of the world. A study in Jordan identified 13 *Mp* isolates by using specific PCR from the milk and nasal cultures [13]. The pathogenic role of *Mp* in small ruminants is also reported by [17] and [18]. In this study *Mp* was only detected in two goats but the pathogenic role of *Mp* in the lungs of goats may not be overlooked.

It is notoriously difficult to isolate the fastidious *Mccp*, the causative agent of CCPP. It has been reported that

CCPP is present in 40 countries but *Mccp* has only been isolated in 13 countries [19]. Certainly the presence of fibrinous pleuropneumonia and pleural fluid in three of the goats and the use of the *Mccp* specific PCR have provided evidence that CCPP is present in Balochistan, Pakistan. One study [20] reported that *Mccp* diagnosis can not be made on clinical symptoms and postmortem examinations alone even though the lesions and large volume of pleural fluid were indicative of CCPP.

These results in the present study have indicated that new strategies are needed to control and limit caprine respiratory diseases in Pishin district. It is also supported by the finding that *Mmm*LC and *Mmc* have not been detected in any of the lung samples of goats in the study area.

We report probably for the very first time, the use of molecular based diagnostic method such as PCR for *Mcc*, *Mp* and PCR-RFLP for *Mccp* in lung samples of goats in Pishin, Balochistan, Pakistan.

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References

1. Nicholas RAJ, Baker SE (1998) Recovery of mycoplasmas from animals. In: Miles RJ, Nicholas RAJ (eds) Mycoplasma protocols. Humana Press, Totowa, pp 37–44
2. Taylor P (1998) Medical significance of Mycoplasmas. In: Miles RJ, Nicholas RAJ (eds) Mycoplasma protocols. Humana Press, Totowa, pp 7–15
3. OIE (2008) Contagious caprine pleuropneumonia. In: Manual of standards for diagnostic tests and vaccines. Office International des Epizooties, Paris, pp 1000–1012
4. Manso-Silván L, Vilei EM, Sachse K, Djordjevic SP, Thiaucourt F, Frey J (2009) *Mycoplasma leachii* sp. *nov*. as a new species designation for *Mycoplasma* sp. Bovine group 7 of Leach, and reclassification of *Mycoplasma mycoides* subsp. *mycoides* LC as a serovar of *Mycoplasma mycoides* subsp. *capri*. Int J Syst Evol Microbiol 59:1353–1358
5. Thiaucourt F, Bolske G (1996) Contagious caprine pleuropneumonia and other pulmonary mycoplasmoses in sheep and goats. Rev Sci Tech 15:1397–1414
6. Bashiruddin JB, Taylor TK, Gould AR (1994) A PCR based test for the specific identification of *Mycoplasma mycoides* subspecies *mycoides* SC. J Vet Diagn Invest 6:428–434
7. Woubit S, Lorenzon S, Peyraud A, Manso-Silvan L, Thiaucourt F (2004) A specific PCR for the identification of *Mycoplasma capricolum* subsp. *capripneumoniae*, the causative agent of contagious caprine pleuropneumonia (CCPP). Vet Microbiol 104:125–132
8. Rubina A, Sarwat NM (2006) Science et changements planétaires. Sécheresse 17(1):203–209
9. Anon (2006) Economic Survey, Government of Pakistan. Fin. Div. Eco. Adv. Wing. Islamabad, Pakistan
10. Tariq MA (1980) Studies on the incidence, epizootology and development of effective vaccines for the control of contagious caprine pleuropneumonia, contagious agalactia in sheep and goats in Balochistan (Annual report), Livestock Department Government of Balochistan (Pakistan)
11. Awan MA, Siddique M, Abbas F, Babar S, Mahmood I, Samad A (2004) Isolation and identification of Mycoplasmas from pneumonic lungs of goats. J App Em Sc 1(1):45–50
12. Shankster S, Ayling RD, Lenfant D, Mercier P, Nicholas RAJ (2002) Development and evaluation of a PCR to detect *Mycoplasma putrefaciens* directly from goats. Res Vet Sci 72(Suppl A):26 (Abstracts)
13. Al-M Waleed, Halablab MA, Abo-Shehada MN, Miles K, McAuliffe L, Nicholas RAJ (2006) Isolation and molecular identification of small ruminant mycoplasmas in Jordan. J Small Rum Res 5:106–112
14. Peyraud A, Woubit S, Poveda JB, Mercier De la Fe C, Thiaucourt F (2003) A specific PCR for the detection of *Mycoplasma putrifaciens*, one of the agents of the contagious agalactia syndrome of goats. Mol Cell Probes 17:1677–1679
15. Perreau P, Breard A (1979) La mycoplasmose caprine a *Mycoplasma capricolum*. Comp Immunol Microbiol Infect Dis 2: 87–97
16. Mercier P, Lenfant D, Poumarat F, Perrin G (2001) Prevalence of mycoplasma infection within French milking caprine herds. In: Poveda JB, Fernandéz A, Frey J, Johansson KE (eds) Mycoplasmas of ruminants: pathogenicity, diagnostics, epidemiology and molecular genetics, vol 5. European Commission, Brussels, Belgium, pp 130–133
17. Damassa AJ, Brooks DI, Holmberg CA, Moe AI (1987) Caprine mycoplasmosis: an out break of mastitis and arthritis requiring the destruction of 700 goats. Vet Rec 120:409–413
18. Adler HE, DaMassa AJ, Brooks D (1980) Caprine mycoplasmosis: *Mycoplasma putrefaciens*, a new cause of mastitis in goats. Am J Vet Res 41:1677–1679
19. Nicholas RAJ (2002) Contagious caprine pleuropneumonia. In: Recent advances in goat diseases. International Veterinary Information Services. Ithaca, NY. www.ivis.org
20. Ozdemir U, Ozdemir E, March JB, Churchward C, Nicholas RAJ (2005) Contagious caprine pleuropneumonia in the Thrace region of Turkey. Vet Rec 156:286–287