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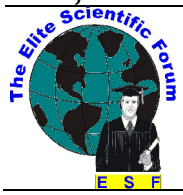

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Prevalence of *Mycoplasma* species by Polymerase Chain Reaction (PCR) directly from the Nasal Swab Samples of Goats

Mohammad Arif Awan[1,*], Ferhat Abbas[1], Masoom Yasin zai[2], Mohammad Masood Tariq[1], Masroor Ahmed Bajwa[1], Mohammad Adnan Attique[1], Zafar Ahmed[1], Nadeem Rashid[1], Majed Rafiq[1] and Mohammad Shafee[1]
[1]Center for Advanced Studies in Vaccinology & Biotechnology (CASVAB), University of Balochistan, Brewery Road, Quetta, Pakistan
[2]Quaid-i-Azam University, Islamabad, Pakistan

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***Corresponding Author:**
arifawan62@yahoo.com

ABSTRACT
The present study highlights the prevalence of *Mycoplasma* species using PCR for *Mycoplasma mycoides* Cluster, *Mycoplasma mycoides* Sub-Cluster, *Mycoplasma capricolum* subspecies *capripneumoniae* (*Mccp*), and *Mycoplasma putrefaciens* (*Mp*) in 1920 nasal swab (DNA) samples of goats from five districts of Balochistan. Overall, 9.2% (n=177) prevalence of *Mycoplasma* species was observed. District-wise prevalence of *Mycoplasma* species indicated statistically significant difference (X^2 =15.78, df = 4, P< 0.0033) with the highest prevalence of 14.1% in goats from Loralai district followed by 9.4% (Pishin), 8.3% (Zhob) and 8.1% (Killa Saifullah), while the lowest prevalence of 6.3% was noted in Quetta District. Of the total 177 (9.2%) nasal swab samples positive for *Mycoplasma* species, the highest prevalence of 69.5% (n=123) was found for *Mmc* followed by 19.2% (n=34) for *Mcc*, whereas the lowest prevalence of 11.3% (n=20) was observed for *Mp* organisms (X^2=266.7, df=3, P<0.0001 indicated statistically highly significant difference). None of the DNA sample was found positive for *Mccp* organisms in specific PCR. The PCR result coupled with restriction fragment length polymorphism (RFLP) profile specifically indicated the presence of *Mmc* in goats in the studied districts of Balochistan. The use of PCR is found promising in the rapid detection of *Mycoplasma* species in the DNA extracted directly from the nasal swab samples of goats in Balochistan, Pakistan.

INTRODUCTION

Members of the class *Mollicutes* inflict a wide range of diseases in both animals and humans, and are generally associated with clinical manifestations such as pneumonia, conjunctivitis, arthritis, abortion and infertility (Nicholas, 2002; McAuliffe et al., 2005). Of the many diseases in goats, the *Mycoplasma* species have been reported for inflicting significant economic losses particularly due to the respiratory diseases (Ozdemir et al., 2005). *Mycoplasma mycoides* cluster is a group of initially six *Mycoplasma* species and subspecies (Cottew et al., 1987), such as *Mycoplasma mycoides* subspecies *mycoides small-colony (Mmm*SC*), Mycoplasma mycoides* subspecies *mycoides large-colony (Mmm*LC*), Mycoplasma mycoides* subspecies *capri* (*Mmc*), *Mycoplasma capricolum* subspecies *capripneumoniae (Mccp), Mycoplasma capricolum* subspecies *capricolum (Mcc)*, and *Mycoplasma* subspecies *bovine group 7* (*BG7*), but recently five species are reported to be the part of this cluster by considering *Mmm*LC and *Mmc* as a single species designated as *Mmc* (Manso-Silvan et al., 2009).

Contagious caprine pleuropneumonia (CCPP) is known as a severe infectious disease of goats caused solely by *Mccp*, and has been reported in most of the Asian and African countries having huge goat population (Rurangirwa and Kinyili, 2000). This disease is an OIE listed disease as it has serious economic impact due to high morbidity and mortality rates, and potential threat to spread to many disease free countries (Woubit et al., 2004). Besides the *Mccp* many of the *Mycoplasma mycoides* Cluster members pose a significant role in respiratory diseases of goats (Thiaucourt and Bolske, 1996). The pathogenic role of *Mmm*LC in pneumonia, arthritis, and mastitis has been well documented (Singh

et al., 2004) in many Asian, African, and European, countries including United States (Smith and Sherman, 1994). Recently *Mycoplasma* species including *Mmc* have been detected in goats using PCR-RFLP besides the biochemical identification of the isolated mycoplasmas in India (Kumar et al., 2011). Further, *Mcc* organism are also known to cause pneumonia and arthritis in goats (Bergonier et al., 1997), and another *Mycoplasma* species such as *Mycoplasma putrefaciens* (*Mp*) has been isolated from the respiratory tract (Radwan et al*.,* 1985) of the goats.

Besides the widely practiced way of isolation and identification to diagnose the mycoplasma diseases (Nicholas et al., 2003), most of the traditional methods are commonly found as time consuming, insensitive and non-specific (Bashiruddin et al., 1994; McAuliffe et al., 2003). This is why *Mccp*, the cause of CCPP, has only been isolated in fewer countries because of difficulties in isolating the organism from the clinical material (Nicholas, 2002).

The Polymerase chain reaction (PCR) has greatly facilitated the rapid diagnosis of mycoplasmas in hours (Nicholas et al., 2003; Grand et al., 2004). Initially a PCR test was reported which could identify members of *Mycoplasma mycoides* cluster followed by restriction fragment length polymorphism (RFLP) with specific restriction endonuclease for the confirmation of *Mccp* (Bascunana et al., 1994). To date, PCR for the detection of *Mycoplasma mycoides* Cluster, PCR-RFLP for *Mycoplasma mycoides* Sub-Cluster members (Bashiruddin et al., 1994), specific PCR-RFLP for *Mccp* (Woubit et al., 2004), and specific PCR for *Mp* (Shankster et al., 2002) have been reported and are widely used in the diagnostic mycoplasmology. It is further reported that the direct detection of *Mccp* in a clinical material may be very useful choice for the confirmation of CCPP outbreaks (Woubit et al., 2004).

Balochistan is the southwestern province of Pakistan, and area wise it is the largest province (Rubina and Sarwat, 2006). Of the 53.8 million goats in Pakistan, 11.8 million goats are present in Balochistan (Anonymous, 2006). These animals are raised under nomadic, transhumant and sedentary production system (Ishaque, 1993). Majority of the goats are prone to infectious diseases, including respiratory mycoplasmosis. Of the many predisposing factors, very cold winters in many areas of Balochistan and poor animal husbandry practices further precipitate the prevailing diseases. There are reports on pleuropneumonia like disease, and CCPP with high morbidity and mortality rates in many parts of Balochistan. Moreover, the continuous migration of small ruminants probably with the respiratory problems from Afghanistan to the adjoining parts of Balochistan may be responsible for increase in the infectious diseases among the goat population (Tariq, 1980; Awan et al., 2009; Awan et al., 2010).

Previous work on mycoplasma diseases in goats in Balochistan, Pakistan has been limited (Tariq, 1980; Awan, 1990, Ahmed, 2005) and couple of research paper has been published on the prevalence of *Mccp, Mcc* and *Mp* in small sample size of goats using molecular test such as PCR (Awan, et al., 2009: Awan et al., 2010) in Balochistan. This extensive study describes for the first time the prevalence of *Mmc* besides the *Mcc*, and *Mp* organisms using PCR directly from the nasal swab samples (n=1920) of goats from five districts of Balochistan.

MATERIALS AND METHODS

All the samples for the extraction of DNA were collected from 1920 nasal swabs of randomly selected goats (with no consideration for age, sex, and breed) from five districts (Quetta, Pishin, Zhob, Loralai and Killa Saifullah) of Balochistan, Pakistan. The present study was carried out at the Center for Advanced Studies in Vaccinology & Biotechnology (CASVAB), University of Balochistan, Quetta, Pakistan.

The DNA was extracted from the nasal swabs (n=1920) by using genomic DNA purification kit (Gentra-Puregene, USA). Briefly the nasal swabs were swirled in 1 ml PBS, and 100ul suspension was used for the extraction of DNA. All the primers used in the present study are shown (Table 1).

The PCR master mix for *Mycoplasma mycoides* Cluster and Sub-Cluster was prepared by following the procedure described by Bashiruddin et al*.,* (1994). The PCR (Thermal cycler, Model # 2720, Applied Biosystem) cycling conditions for *Mycoplasma mycoides* Cluster and Sub-Cluster were similar (Bashiruddin et al*.,* 1994). Further the PCR master mix for *Mycoplasma capricolum* subspecies *capripneumoniae (Mccp) and Mycoplasma putrefaciens (Mp)* was prepared by following the method as described by Woubit et al*.,* (2004) and Shankster et al*.,* (2002) respectively.

Two percent (2%) Agarose (Vivantis-USA) gel was used for gel electrophoresis (35 minutes at 100 Volts). The gel slab was observed for PCR product (band) by the gel documentation system (Dolphin-View, Wealtec-USA).

The presence of *Mcc* organisms in all the DNA samples collected from nasal swab samples of the goats was based on the results of DNA samples positive in *Mycoplasma mycoides* Cluster PCR, negative in *Mycoplasma mycoides* Sub-Cluster, *Mccp*, and *Mp* PCR tests.

The RFLP for the validation of *Mycoplasma mycoides* Sub-Cluster PCR (amplicon) product was performed (Bashiruddin et al*.,* 1994). For the RFLP, *Vsp1* restriction endonuclease (Vivantis, USA) was used. The digested PCR product was electrophoressed with 3%

Table 1: Sequence of primers (Oligonucleotides) used in PCRs for the identification of *Mycoplasma* species

PCR for *Mycoplasma* species	Primers	Sequence (5'-3')	Orientation
Mycoplasma mycoides Cluster [1]	MC323	TAG AGG TAC TTT AGA TAC TCA AGG	Forward
	MC358	GAT ATC TAA AGG TGA TGG T	Reverse
Mycoplasma mycoides Sub-Cluster [1]	MM450	GTA TTT TCC TTT CTA ATT TG	Forward
	MM451	AAA TCA AAT TAA TAA GTT TG	Reverse
Mycoplasma capricolum subspecies *capripneumoniae (Mccp)* [2]	Mccp-spe-F	ATC ATT TTT AAT CCC TTC AAG	Forward
	Mccp-spe-R	TAC TAT GAG TAA TTA TAA TAT ATG CAA	Reverse
Mycoplasma putrefaciens [3]	SSF1	GCG GCA TGC CTA ATA CAT GC	Forward
	SSR1	AGC TGC GGC GCT GAG TTC A	Reverse

[1]Bashiruddin et al., 1994; [2]Woubit et al., 2004; [3]Shankster et al., 2002

agarose gel in TAE buffer and seen in the gel document system. Further as none of the DNA sample extracted from nasal swab of goats was positive in *Mccp* specific PCR, therefore *Mccp*-RFLP was not performed.

Statistical analysis

Proportions and Chi-square test were used to analyze the data. Chi-square test was used to test the difference (P <0.05 was considered significant) in the prevalence of mycoplasma organisms in goats in DNA samples by districts and *Mycoplasma* species. The Graphpad Prism 5 for Windows version 5.04 was used to analyze the data statistically.

RESULTS

The *Mycoplasma mycoides* Cluster members (Figure 1), *Mycoplasma mycoides* Sub-Cluster members (Figure 2) and *Mycoplasma putrefaciens* (Figure 3) are identified by the presence of 1500bp, 574bp, and 800bp PCR products respectively. None of the DNA sample from nasal swabs of the goats was found positive in *Mccp* specific PCR (Figure 4). The RFLP results for the PCR product (574bps) of *Mycoplasma mycoides* Sub-Cluster members (*Mmc* and *Mmm*SC) yielded three bands (fragments) of 230, 178, and 153bps specifically for *Mmc* when digested with *Vsp1* (Figure 5). None of the PCR product was observed with two bands of 379bps and 178bps specific for *Mmm*SC in RFLP.

The results for the prevalence of *Mycoplasma* species by PCR in the nasal swabs of goats are shown (Table 2). Over all of the total nasal swab samples (n=1920) from goats 9.2% prevalence (n=177) for total *Mycoplasama* species was observed. The prevalence of total *Mycoplasma* species in goats among districts (study sites) indicated highest rate of 14.1% (n=54) in nasal swab samples of goats in Loralai district followed by 9.4% (n=36) in Pishin district while the lowest rate of 6.3% (n=24) was noticed in Quetta district. Statistically significant difference (X^2 = 15.78, df = 4, P < 0.0033) was observed.

The prevalence of *Mmc* (Table 2) indicated highest rate of 10.2% (n=39) in Loralai district followed by 6.3% (n =24) in Zhob and Killa Saifullah districts. Conversely the lowest prevalence of 3.9% (n=15) was observed in Quetta district. Statistically significant difference (X^2 = 13.6, df=4, P=0.0087) was noticed in the prevalence of *Mmc* by study sites.

The prevalence of *Mcc* among goats by study districts (Table 2) indicated highest rate of 2.6% (n=10) in Loralai district followed by 2.1% (n=8) in Pishin district. Conversely the lowest prevalence of 1.3% (n = 5) was observed in Zhob and Killa Saifullah districts. Statistically non-significant difference (X^2 = 2.815, df = 4, P= 0.5893) was noticed in the prevalence of *Mcc* by study sites.

The prevalence of *Mp* among goats by study districts (Table 2) indicated highest rate of 1.8% (n=7) in Pishin district followed by 1.3% (n=5) in Loralai district. Conversely the lowest prevalence of 0.5% (n=2) was observed in Killa Saifullah district. Statistically non-significant difference (X^2 =4.042, df=4, P=0.4003) was noticed in the prevalence of *Mp* by study sites.

The prevalence of *Mycoplasma* species in positive nasal swab samples (n=177) by PCRs indicated highest frequency of 123 (69.5%) for *Mmc* followed by 34 (19.2%) for *Mcc* while the lowest frequency of 20 (11.3%) for *Mp* was observed. None of the nasal swab samples of goat was found positive by *Mccp* specific PCR (Table 2). Statistically highly significant difference (X^2 = 266.7, df=3, P< 0.0001) was observed.

DISCUSSION

Caprine pleuropneumonia and other respiratory symptoms associated with the *Mycoplasma* species are common in Balochistan. Regardless of the traditional diagnostic means, which have been used for infectious diseases including mycoplasmosis, are time consuming, non-specific and laborious and above all the isolation of *Mccp* is considered as a difficult task.

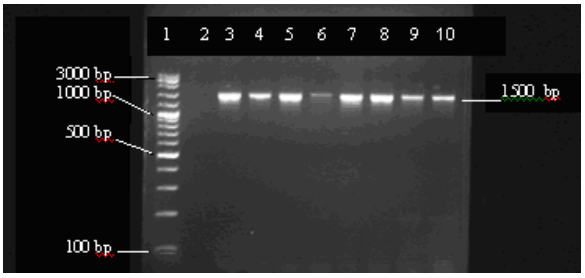

Fig. 1: PCR profile of *Mycoplasma mycoides* Cluster members (amplicon size of 1500bp is positive) obtained from the nasal swab samples of goats. Lane 1: Molecular ladder; lane 2: –ve control; lanes 3-9 samples positive for *M. mycoides* Cluster members; lane 10: *Mycoplasma mycoides* subspecies *capri (Mmc*) + ve control.

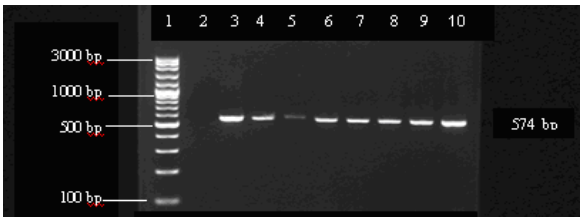

Fig. 2: PCR profile of *M. mycoides* Sub-Cluster members (amplicon size of 574bp is positive) obtained from the nasal swab samples of goats. Lane 1: Molecular ladder; lane 2:–ve control; lanes 3-9 positive samples for *M. mycoides* Sub-Cluster members; lane 10: *Mmc* + ve control

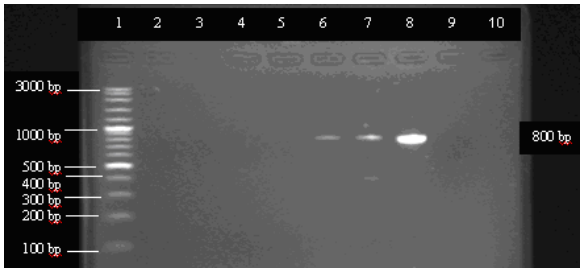

Fig. 3: PCR profile of *Mycoplasma putrefaciens* (*Mp*) (amplicon size of 800bp is positive) obtained from the nasal swab samples of goats. Lane 1: Molecular ladder; lane 2: –ve control; lanes 3-5 negative samples for *Mp*; lanes: 6-7 +ve samples for *Mp*; lane 8: *Mp* +ve control; lanes 9 and 10 empty wells.

It requires a special laboratory facility and excellent expertise in dealing with such fastidious microorganisms. Previously, limited reports on the isolation and identification of *Mmc* from the lung tissues of goats by using the classical biochemical and

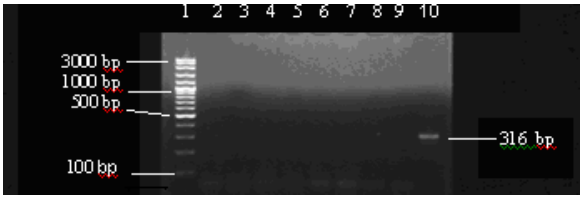

Fig. 4: PCR profile of *Mycoplasma capricolum* subspecies *capripneumoniae* (*Mccp*) (amplicon size of 316bp is positive) for nasal swab samples of goats from Quetta district in Balochistan. Lane 1: Molecular ladder; lane 2:–ve control; lanes 3-9 negative samples for *Mccp*; lane 10: *Mccp* + ve control.

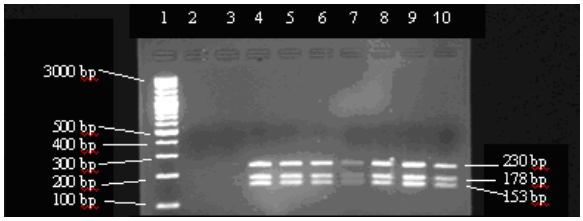

Fig. 5: RFLP profile for the PCR product (574bp) of *M. mycoides* Sub-Cluster members (fragments of 230,178, and 153bp are specific for *Mmc* after the digestion of 574bp PCR product with *Vsp*1 restriction endonuclease) obtained from the nasal swab samples of goats from Loralai district in Balochistan. Lane 1: Molecular ladder; lane 2:–ve control; lanes 3-9 positive samples for *Mmc* (lane 3 faint fragments); lane 10: *Mmc* +ve control

serological techniques in Balochistan have been reported (Tariq, 1980; Awan, 1990; Awan et al., 2004). There are reports on the prevalence of *Mycoplasma* species such as *Mccp*, *Mp* and *Mcc* using DNA from lung tissue of pleuropneumonia suspected goats (n=30) in Pishin district of Balochistan (Awan et al., 2009: Awan et al., 2010). The present study for the first time highlights the prevalence of *Mmc, Mp* and *Mcc* by PCR test directly on the nasal swab samples (DNA) of the randomly selected goats (n=1920) from five districts in Balochistan.

The PCR has been reported as a qualitative and quantitative diagnostic test for mycoplasmas. In the present study all the PCR tests used were qualitative. The presence of a specific PCR amplicon (band) indicated the presence of infection or disease in goats with the *Mycoplasma* species. The specificity of the PCR for *Mycoplasma mycoides* sub-cluster members was further validated by RFLP using specific restriction endonuclease. The PCR and RFLP profiles obtained in the present study were similar to those as reported previously (Bashiruddin et al., 1994).

Table 2: Prevalence of *Mycoplasma* species in goats in five districts of Balochistan

				1	2	3	4
Districts (Study sites)	No. of nasal swab samples of goats	+ve for total *Mycoplasma* species (1+2+3+4) n (%)	+ve for *Mycoplasma mycoides* Cluster PCR[1] (1+2+3) n (%)	+ve for *Mycoplasma mycoides* Sub-Cluster PCR-RFLP[1a] n (%)	+ve for *Mccp* specific PCR[2,4] n	+ve for *Mcc*[*] n (%)	+ve for *Mp* specific PCR[3] n (%)
Quetta	384	24[b] (6.3)	21	15[c] (3.9)	0	6[d] (1.6)	3[e] (0.8)
Pishin	384	36[b] (9.4)	29	21[c] (5.5)	0	8[d] (2.1)	7[e] (1.8)
Zhob	384	32[b] (8.3)	29	24[c] (6.3)	0	5[d] (1.3)	3[e] (0.8)
Loralai	384	54[b] (14.1)	49	39[c] (10.2)	0	10[d] (2.6)	5[e] (1.3)
Killa Saifullah	384	31[b] (8.1)	29	24[c] (6.3)	0	5[d] (1.3)	2[e] (0.5)
Total	1920	177 (9.2)	157 (2.7)	123[f] (2.1)	0[f]	34[f] (0.6)	20[f] (0.3)

[1]Bashiruddin *et al*., 1994, [2] Woubit *et al*., 2004, [3] Shankster *et al*., 2002; [4]RFLP was not carried out as none of the sample was positive in *Mccp* specific PCR; [*]Samples were identified as *Mcc* as these were found positive for *Mycoplasma mycoides* cluster PCR, negative for *Mycoplasma mycoides* sub-cluster, *Mccp*, and *Mp* PCRs; [a] RFLP yielded 3 bands specific for *Mycoplasma mycoides* subspecies *capri (Mmc);* [b] $X^2 = 15.78$, df = 4, p-value < 0.0033 (District-wise highly significant difference in the prevalence of mycoplasmas in goats); [c] $X^2 = 13.6$, df = 4, p-value =< 0.0087 (highly significant difference in the prevalence of *Mmc* among districts); [d] $X^2 = 2.815$, df = 4, p-value =0.5893 (non- significant difference in the prevalence of *Mcc* among districts); [e] $X^2 = 4.042$, df = 4, p-value =0.4003 (non- significant difference in the prevalence of *Mp* among districts); [f] $X^2 = 266.7$, df = 3, p-value < 0.0001 (*Mycoplasma* species-wise highly significant difference among goats)

In the present study the comparatively higher prevalence of *Mmc* than the *Mcc* and *Mp* in nasal swabs from the randomly selected goats in five districts of Balochistan is critical (Table 1). The higher prevalence of *Mmc* in the goats is alarming in all the studied districts. This is also supported by the studies (Awan, unpublished data) in which the researcher has indicated the presence of respiratory symptoms, gross-pathological lesions consistent with the mycoplasma oriented respiratory disease, and isolation and identification of *Mycoplasma* species such as *Mmc*, *Mcc*, and *Mp* by the biochemical, serological and PCR-RFLP tests. Similarly higher prevalence (8.3%) of *Mycoplasma* species including *Mmc* using PCR have been reported in goats in Western India (Kumar et al., 2011). Besides the traditional diagnostic tests for the mycoplasma infections the use of molecular biological tools have also been reported. *Mycoplasma mycoides* subspecies *capri* (*Mmc*) was identified as the cause of a CCPP like disease in goats by the PCR test (Hernandez et al., 2006). The pathogenic role of *Mmc* is well reported in the literature and in the present study the prevalence of 2.1% (n=123) *Mmc* in the nasal swab samples of the randomly selected goats in 5 districts in Balochistan can not be ignored. Previously it is reported that *Mmc* (Formerly *MmmLC)* affects the goats population over a wide range of countries (DaMassa et al., 1992) and is suspected, whenever pleuropneumonia, pneumonia and contagious agalactia are reported (Cottew, 1979). The severe and very acute cases of *Mmm*LC infections in goats are reported with the death of animals without showing any apparent clinical sign and symptom (Smith and Sherman, 1994). Moreover mastitis, pneumonia and arthritis have also been observed in the goats affected with *Mmm*LC and *Mmc* organisms during a disease (Kumar et al., 1994). In a microbiological study, Indian strain of *Mmm*LC is isolated from a goat with arthritis (Singh et al., 2004). There have been little reports on the natural and experimental cases of *Mmc* pathogenecity.

In the present study none of the nasal swab samples is found positive in *Mccp* specific PCR. In another study more interestingly of the CCPP suspected goats (n=30) from Pishin district, Balochistan, Pakistan only lung samples from pleuropneumonia suspected goats (n=3) were positive for *Mccp* by the *Mccp* specific PCR and RFLP (Awan et al*,* 2010). Further, there is a consistent influx of healthy and diseased goats from the adjoining areas of Afghanistan into the border areas in Balochistan. These findings suggest that the diagnosis of CCPP under field conditions may not be straightforward (Wesonga et al., 2004). The CCPP has been reported in 40 countries, but due to the fastidiousness of *Mccp*, its isolation is reported to be a difficult task, this is why *Mccp* has only been isolated from goats only in 13 countries (Nicholas, 2002).

The comparatively low prevalence of *Mcc* and *Mp than Mmc* by the PCR in the nasal swabs of the goats can not be ignored (Table 1). All the samples collected for this study were statistically representative (95% CI, 5% absolute precision with 50% expected prevalence of mycoplasma disease). The results therefore suggest that

the prevalence of mycoplasma inflicted infection/ disease in goats may be equal or more than 50% in districts in Balochistan. Stringent surveillance studies are required to further highlight the prevalence of *Mycoplasma* species in goats throughout the Balochistan in order to plan for an effective control of the prevailing mycoplasma diseases. *Mcc* has been a known pathogen for goats. The rationale for the molecular identification of *Mcc* in the present study included all positive samples in *Mycoplasma mycoides* Cluster PCR, and all negative samples in *Mycoplasma mycoides* Sub-Cluster, *Mccp*, and *Mp* specific PCRs. However, the use of *Mcc* specific PCR could not be used to validate the detection of *Mcc* in the nasal swab samples from goats in the present study. The prevalence of *Mcc* was found low in the present study, and is also supported by the research in which *Mcc* was detected in milk and nasal cultures of goats in Jordan, using PCR assays (Al-Momani et al., 2006). Moreover, the presence of *Mycoplasma Bovine group 7*, one of the members of the *Mycoplasma mycoides* cluster, is excluded from all the nasal swab samples (n=1920) in the present study as this species is generally not prevalent in goats.

In the present study, the PCR based detection of *Mp* in the nasal swabs can not be ignored and extensive studies are required to explore its isolation and characterization as well as reproduction of an experimental disease in the susceptible goats. Though the pathogenic potential of *Mp* is not well established and looks variable particularly in the caprine respiratory diseases. Contrary to this, *Mycoplasma putrefaciens* is also reported as one of the causative agents of CA syndrome (Manso-Silva´n et al., 2009). Besides mastitis it has also been reported to cause septicaemia in kids and arthritis in adults (Peyraud et al., 2003). Further, *Mp* can be isolated from animals with or without clinical signs (Mercier et al., 2001) suggesting a carrier status. Further in a study in Jordan 13 *Mp* isolates have been identified by using specific PCR from the milk and nasal cultures (Al-Momani et al., 2006). The pathogenic role of *Mp* in small ruminants is also reported (Adler et al., 1980; DaMassa et al., 1987).

Overall it can be concluded that the goats in the five studied districts of Balochistan are having higher prevalence of *Mmc* followed by *Mcc* and *Mp*. *Mccp* could not be detected. The *Mycoplasma* isolates are required to be further explored for their pathogenic potential in order to prepare an effective vaccine against prevailing mycoplasmal diseases characterized with respiratory and arthritic manifestation in goats in Balochistan.

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