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Short communication

Isolation rate and antimicrobial susceptibility profiles of *Mycoplasma mycoides* subspecies *capri* field isolates from sheep and goats in Pakistan

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ABSTRACT

Mycoplasmosis, the respiratory infection, is a well-established cause of heavy economic losses in sheep and goats farming. The occurrence and antibiotics susceptibility profiles of clinical isolates of *Mycoplasma mycoides* subsp. *capri* (*Mmc*) from suspected cases of contagious caprine pleuropneumonia (CCPP) were investigated in small ruminants of three climatic- southern, central and northern- regions of Khyber Pakhtunkhwa province of Pakistan. Specie specific-PCR for *Mmc* confirmed 24% (198/825) isolates with significantly ($P < 0.001$) higher number (83/275; 30.18%) recovered from the northern-, followed by southern- (68/275; 24.73%) and central-regions (47/275; 17.09%), respectively. Subsequently, a total of 54 PCR-confirmed isolates (18 from each zone) were subjected to antibiogram assay. Five different common antimicrobial agents including ceftiofur, enrofloxacin, gentamycin, oxytetracycline and tylosin were tested by disc diffusion and broth microdilution methods The antibiotic susceptibility profiles of *Mmc* isolates indicated that enrofloxacin exhibited the highest ($P < 0.001$) *in vitro* antibacterial activity with a maximum zone of inhibition (19 ± 0.71 mm) and the lowest MIC (0.001 ± 0.0002 mg/mL) amongst the tested antimicrobial agents, followed by gentamycin (11 ± 0.45 mm and 0.01 ± 0.001 mg/mL zone of inhibition and MIC, respectively). All these isolates were found resistant against tylosin, oxytetracycline and ceftiofur sodium: antibiotics that are favored in clinical cases of CCPP. This study concludes a high occurrence of *Mmc* in small ruminants during infection of CCPP, and increased resistance rate against commonly used antibiotics.

1. Introduction

Ruminant mycoplasmosis, an important respiratory tract infection considered to cause heavy economic losses, is often reported from countries of Africa and Asia including Pakistan (Sadique et al., 2012; Shahzad et al., 2013). Pakistan is ranked 3rd in goat- and 12th in sheep-production and sheep and goat sector contributes considerably in the overall economy of the country (Economic Survey, 2013). Mycoplasmosis, a principal cause of acute respiratory syndrome that usually terminated in chronic complications, lead to huge economic losses in the form of diminished production, treatment cost, high mortality and decreased export, and is considered as serious threat for the productivity of sheep and goats (Nicholas et al., 2008; OIE, 2017).

Contagious caprine pleuropneumonia (CCPP), a respiratory disease syndrome, is caused by a group of six closely related microorganisms known as *Mycoplasma mycoides* (*Mm*) cluster. *Mycoplasma mycoides* subsp. *capri* (*Mmc*) is one of the most prevalent pathogenic species among the *Mm* cluster (Manso-Silvan et al., 2007). *Mmc* could cause CCPP in addition to complications like arthritis, mastitis, kerato-conjunctivitis (altogether known as contagious agalactia), urogenital infection and occasionally causing abortion in the pregnant ewes (Mondal et al., 2004; Nicholas et al., 2008). CCPP commonly occurs in septicemic form accompanied by high morbidity and mortality (Sadique et al., 2012), and is listed in the OIE-listed diseases, infection and infestations 2017 (OIE, 2017). Although, CCPP is generally caused by *Mycoplasma capricolum* subsp. *capripneumoniae* (*Mccp*), which affects thoracic cavity mainly (OIE, 2017); however, in Asia and Africa, *Mmc* has been reported frequently associated with CCPP (Gelagay et al., 2007; Mondal et at., 2004; Sadique et al., 2012; Shahzad et al., 2012). The disease is contagious in nature, which rapidly spreads among animals by direct contact through oculonasal discharge, excretion from wound, open joints, urine and faeces (OIE, 2017).

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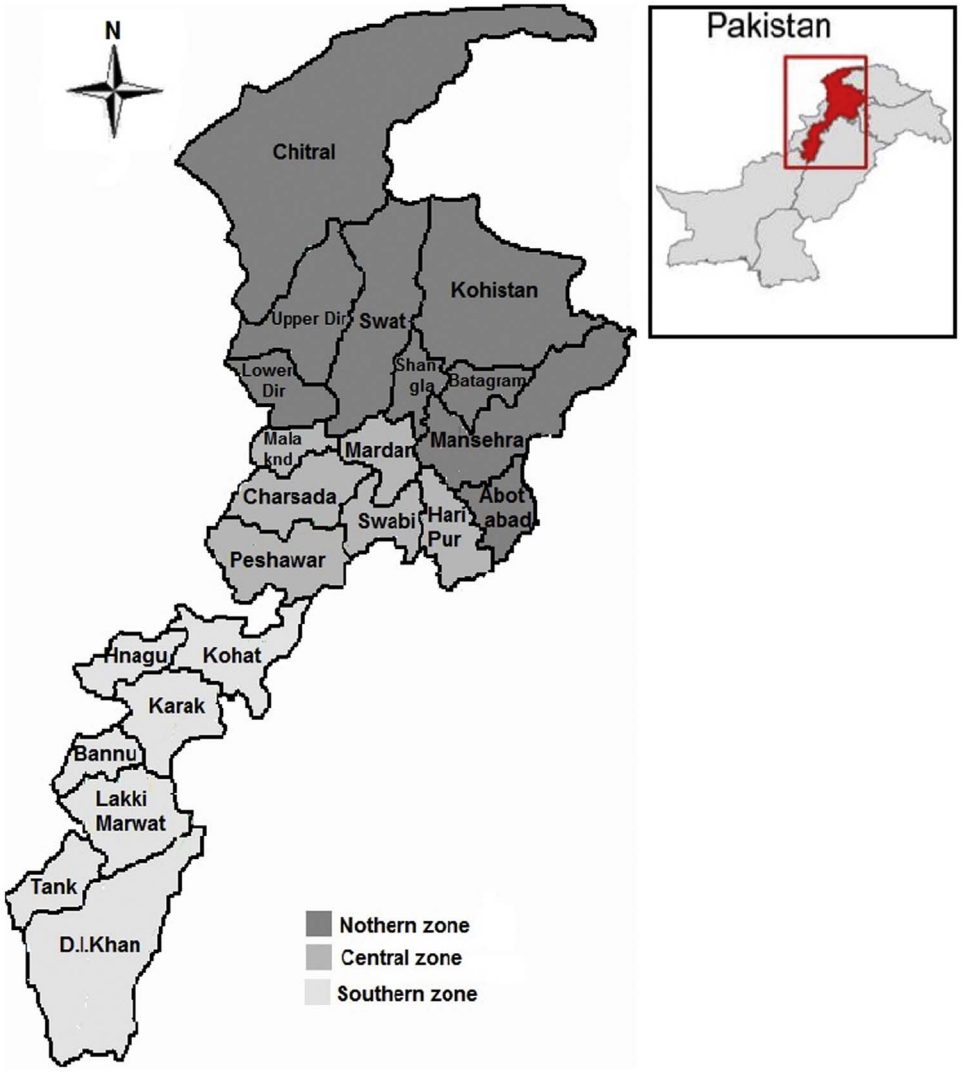

Fig. 1. Map of Khyber Pakhtunkhwa province of Pakistan, showing the three regions i.e. northern, central and southern, which were sampled for the detection of *Mycoplasma mycoides* subsp. *capri*.

Table 1
Isolation rate of *Mycoplasma mycoides* subsp. c*apri* in suspected of contagious caprine pleuropneumonia in small ruminants.

Climatic Zones		Status on PCR		Total	Chi-square	*P*-value
		Positive	Negative			
Northern	Number	83	192	275	13.03	0.001
	% within zone	30.18	69.81	100.0		
Central	Number	47	228	275		
	% within zone	17.09	82.91	100.0		
Southern	Number	68	207	275		
	% within zone	24.73	75.27	100.0		
Total	Number	198	627	825		
	% within zone	24.0	76.0	100.0		

CCPP is usually treated with antibiotics like enrofloxacin, gentamycin, kanamycin, oxytetracycline, tylosin and third generation cephalosporins such as ceftiofur. However, due to indiscriminate use of these antimicrobials drug resistance have been recently developed with cases of therapeutic failure (Citti and Blanchard, 2013; Scott and Menzies, 2011). Therefore, the current study was carried out with objectives to investigate the occurrence and antimicrobial susceptibility profiles of clinical isolates of *Mmc* from diverse localities of Khyber Pakhtunkhwa.

2. Material and methods

2.1. Sampling, isolation and identification of Mycoplasma mycoides subsp. capri (Mmc)

The clinical *Mmc* isolates were recovered from sheep and goats suffering from respiratory complications from three different climatic regions *viz.*, northern, central and southern of Khyber Pakhtunkhwa (KPK), Pakistan during December 2014 to May 2016 (Fig. 1). A total of 825 clinical samples, including nasal (n = 450), tracheal swabs (n = 300) and lungs tissue (n = 75) were collected from animals suspected of contagious caprine pleuropneumonia (CCPP). Of these samples, 412 and 413 were collected from sheep and goats, respectively. From each zone, 275 samples, including nasal = 150, tracheal = 100 and lungs = 25, were collected. Samples were collected with sterile swabs, transferred into Difco™ PPLO broth (Becton Dickinson, Sparks, MD), and transported in ice box to our Mycoplasma reference laboratory, Faculty of Animal and Veterinary Science, University of Agriculture, Peshawar. All collected samples were incubated in anaerobic incubator (New Brunswick, Galaxy 48-S, UK) with 5% CO_2 at 37 °C for 3–7 days. The incubated test tubes were examined daily for presence of mass turbidity, whirling movement and change in color. The positive growth sample was sub-cultured on Difco™ PPLO agar for the appearance of nipple like or fried egg shape typical mycoplasma colonies. The desired colonies were sub-cultured three times to obtain pure culture