RESEARCH ARTICLE

Molecular Detection of Bacterial Pathogens Directly from the Nasal Swabs and Lung Tissue of Sheep and Goats

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Abstract

Respiratory infections of sheep and goats cause heavy morbidity and mortality, leading to huge economic losses. Conventional methods of diagnosis that include isolation and identification of incriminating microbes are time-consuming and fraught with logistic challenges. Direct detection of incriminating microbes using molecular tools is gaining popularity in clinical, microbiological settings. In this study, a total of 50 samples (44 nasal swabs and 6 lung tissues) from sheep and goats were screened for the detection of different bacterial species by *in vitro* amplification of genus or species-specific genes. *Histophilus somni* was detected in 2% goat samples, *Trueperella pyogenes* in 20% goat nasal swabs, whereas 22% goat nasal swab samples were found positive for *Mycoplasma* spp. None of the samples from sheep was detected positive for *H. somni*, *T. pyogenes*, *Mycoplasma* spp. Similarly, all samples, irrespective, whether from sheep or goats, showed negative results for *Pasteurella multocida*, *Mannheimia haemolytica,* and *Corynebacterium pseudotuberculosis*.

Keywords: Goat, *Histophilus somni*, Mycoplasma, Respiratory infections, Sheep, *Trueperella pyogenes*.

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Introduction

Infectious diseases are a common cause of economic losses to small ruminants, mainly sheep and goat husbandry. To enable sheep and goat husbandry viable and sustainable amongst rural people, the development and use of techniques for early and accurate diagnosis hold prime importance (Chakraborty *et al*., 2014). Respiratory diseases of small ruminants are multifactorial (Lacasta *et al*., 2008), and there are multiple etiological agents responsible for the respiratory disease complex. The most common bacterial causes of respiratory infections are *P. multocida*, *Mannheimia hemolytica*, *Mycoplasma* spp., *Corynebacterium pseudotuberculosis, Histophilus somni,* and *Trueperella pyogenes*. These bacteria are commonly found in the respiratory tract of sheep and goats and induce morbidity and mortality under immunosuppressive conditions. Mycoplasma infections cause indirect economic losses as a result of emaciation, delayed market weight, and infertility, owing to the sub-acute or chronic pneumonia, especially in small ruminants, which are of great importance in rural development. A major health problem of small ruminants is pneumonia/ pleuropneumonia, which may be caused by Mycoplasma species alone or in conjunction with other microbes (Adehan *et al*., 2006). The isolation of these bacterial species is time-consuming and requires different media and laboratory conditions. The objective of this study was to fill up the gap in the literature in the detection of bacterial pathogens directly from the nasal swabs and lung tissue of sheep and goats through PCR technology.

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Materials and methods

Sampling and Transportation

A total of 50 samples comprising of 44 nasal swabs from 15 sheep and 29 goats; and 6 lung tissues from 2 sheep and 4 goats having respiratory disease symptoms (nasal discharge, sneezing, coughing, and respiratory distress) were collected in PBS and transported on ice to the laboratory. All samples were stored at 4ºC until processed.

Molecular Detection through In Vitro Amplification

All samples were processed for DNA extraction as per the Phenol Chloroform Isoamyl alcohol (PCI) protocol given by Sambrook *et al*. (1989). The polymerase chain reaction was performed for the detection of different bacterial species

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by using genus-specific or species-specific primers, as listed in Table 1. The PCR reaction was carried out in a reaction volume of 25 µl in the GeneAmp PCR system 9700 (Applied Biosystems, USA) thermocycler. PCR reaction was prepared as per Table 2. PCRs were run as per the conditions given in Table 3. The amplified products were detected by staining with ethidium bromide (0.5 mg/mL) after electrophoresis at 100 V for 30 minutes in 1% agarose gel.

Results and discussion

Out of 50 samples (44 nasal swabs and 6 lung tissues), only one (2%) goat nasal swab sample was positive for *H. somni* and yielded a 400 bp amplicon, as shown in Fig.1(a). Romero *et al*. (2011) reported 2.3% isolation of *H. somni* from goat nasal exudate and confirmed *H. somni* colonies by 16S rRNA PCR. Out of 50 samples, 10 (20%) goat nasal swab samples were found positive for *T. pyogenes* and amplified an expected 270 bp product, as shown in Fig.1(b). Hadimli *et al*. (2011) identified 7.2% *T. pyogenes* by means of PCR using *plo* gene from lung tissues, liver and liver abscesses of sheep. Ribeiro *et al*. (2015) suggested that pyolisin (*plo*) is a potent cytolisin related to tissue damage and is considered the primary virulence factor in *T. pyogenes*. Globally, current research efforts are focused on innovations that enhance the application of *in vitro* amplification techniques for direct detection of pathogens from clinical samples. This approach has several advantages over cultural techniques, particularly for slow-growing or non-cultivable bacteria.

Surprisingly, all samples screened for *Pasteurella multocida*, *Mannheimia haemolytica*, and *Corynebacterium pseudotuberculosis* gave negative results in their respective PCRs. *P. multocida* and *M. haemolytica* are usually present

Table 1: Details of primers used for the detection of bacterial species.

Primers	*Sequence (5'-3')*	*Bacteria*	*Product size (bp)*	*Specificity*	*Reference*
KMT1SP6 F	GCTGTAAACGAACTCGCCAC	Pasteurella multocida	460	*kmt1 gene*	Townsend *et al.* (1997)
KMT1T7 R	ATCCGCTATTACCCAGTGG				
Rpt2 F	GTTTGTAAGATATCCCATTT	*Mannheimia haemolytica*	1022	*Rpt2 gene*	Ryan and Lo (1998)
Rpt2 R	CGTTTTCCACTTGCGTGA				
16S rRNA F	ACCGCACTTTAGTGTGTGTGTG	*Corynebacterium pseudotuberculosis*	815	16S rRNA	Centinkaya *et al.* (2002)
16S rRNA R	TCTCTACGCCGATCTTGTAT				
HS F	GAAGGCGATTAGTTTAAGAG	*Histophilus somni*	400	16S rRNA	Angen *et al.* (1998)
HS R	TTCGGGCACCAAGTRTTCA				
plo F	GGCCCGAATGTCACCGC	*Trueperella pyogenes*	270	*plo gene*	Billington *et al*. (2002)
plo R	AACTCCGCCTCTAGCGC				
GPO3 F1	GGGAGCAAACAGGATTAGATACCCT	*Mycoplasma* genus specific	288	16S rRNA	Kuppeveld *et al*. 1992
MGSO R	TGCACCATCTGTCACTCTGTTAACCTC				

Table 2: Concentration and quantity of various components used in PCR reaction mixture

Ingredients	*Quantity (µl)*	*Final concentration*
Colored GoTaq PCR buffer 5X	5	1X
$MgCl_2$ (25 mM)	2	2 mM
dNTPs (10 mM)	0.5	0.2 mM
Forward primer (10 µM)	1	0.4 µM
Reverse primer (10 µM)	1	0.4 µM
Template	5	100 ng
GoTaq DNA polymerase	0.3	0.06 U
Nucleus free water	10.2	
Total	25.0	

Table 3: Different PCR conditions

		Cycle				
Primer set	*Initial denaturation temp-time*	*Denaturation temp-time*	*Annealing temp-time*	*Extension temp-time*	*Final extension temp-time*	*Cycles*
KMT1SP6-F KMT1T7 R	95ºC- 4 min	95ºC- 1 min	55ºC- 1 min	72ºC- 1 min	72ºC- 9 min	30
Rpt2 F & R	95ºC- 3 min	95ºC- 1 min	48ºC- 1 min	72ºC- 30 sec	72ºC- 5 min	35
16S rRNA F & R	94ºC- 5 min	94ºC- 1 min	55ºC- 1 min	72ºC- 2 min	72ºC- 5 min	30
HS F & R	94ºC- 3 min	94ºC- 1 min	55ºC- 1 min	72ºC- 1 min	72ºC- 5 min	35
plo F & R	95ºC- 5 min	95ºC- 30 sec	55ºC- 45 sec	72ºC- 50 sec	72ºC- 5 min	30
GPO3 F1 & MGSO R	94ºC- 5 min	94ºC- 30 sec	55ºC- 30 sec	72ºC- 30 sec	72ºC- 5 min	30

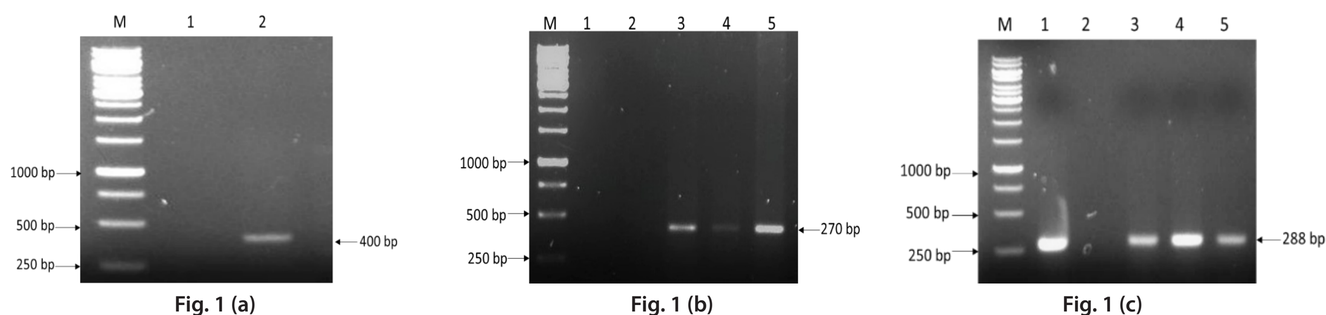

Fig. 1: Agarose gel electrophoresis

(a) Detection of *Histophilus somni* based on 16S rRNA PCR Lane M- 1kb ladder, lane 1- negative PBS control, lane 2- positive *H. somni* sample

(b) Detection of *T. pyogenes* based on plo PCR Lane M- 1kb ladder, lane 1-negative PBS control, lane 2-negative sample, lane- 3 to 5 positive *T. pyogenes* samples

(c) Detection of *Mycoplasma* spp. based on 16S rRNA PCR, Lane M-1kb ladder, lane 1-positive DNA control, lane 2- negative PBS control, lane- 3 to 5 positive samples

in the tonsils and retropharyngeal lymph nodes of carrier animals. *Corynebacterium pseudotuberculosis*, on the other hand, causes mostly caseous lymphadenitis in sheep and goats, and therefore, an appropriate sample for isolation or detection should be infected lymph nodes rather than nasal swabs. Despite all the conditions, tissue tropism, and favored host niches, it was expected to have these species detected in nasal swabs through PCR. However, negative results may arise from the inherent limitation of less sensitivity of conventional PCR owing to the minimal amount of the target DNA. In this study, the PCR using GPO3 F1 and MGSO R primers was performed on 50 samples. Out of 50, 11 (22%) goat nasal swabs were found positive and amplified 288 bp product, as shown in Figure 1(c). Kong *et al*. (2001) detected mollicute DNA in 60 of 70 cell lines using GPO-3 & MGSO primers and concluded that PCR is more sensitive than culture for detecting mycoplasma. Kumar *et al*. (2011) reported that the Mycoplasma having a 16S rRNA gene allowed the identification of variable regions with both genus and species-specific primers to identify the particular species of mycoplasma cluster. The current study recorded the highest prevalence of *Mycoplasma* spp., followed by *T. pyogenes* and *H. somni,* respectively. The important opportunistic pathogen, *P. multocida* was not encountered in any of the processed samples from sheep and goats understudy even though this department has been routinely detecting *P. multocida* from calves, buffaloes, and rabbits. The prevalence rates of these organisms, along with other bacteria and viruses involved in respiratory infections, should be studied using a combination of techniques that involve conventional microbiology, PCR, and real-time PCR in both diseased and healthy animals. This would give a clear picture of the involvement of these microbes in health and disease.

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