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Diagnosis of Etiological Agent on Caprine *Mycoplasma pneumonia* in Chongqing China

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Abstract: One mycoplasma was isolated from a lung of goat. The strain was identified as *Mycoplasma mycoides* subsp. *Mycoides* by PCR.

Key words: Caprine, mycoplasmal pneumonia, *Mycoplasma mycoides* subsp. *Mycoides*, strain, lung of goat

INTRODUCTION

Infectious pleuropneumonia, commonly known as black lung disease is caused by mycoplasma, cough, runny nose liquid, dyspnea, progressive emaciation and pulmonary interstitial hyperplastic inflammation characterized contact (Pan *et al*., 2002) highly infectious, morbidity and mortality for the characteristics of an infectious disease (Zhang *et al*., 2007a), its main harm is caused by the sheep are slow growing, body condition falls, the disease of the other secondary disease; complications make it seriously affected sheep industry economic benefits.

Sheep mycoplasmal pneumonia epidemic in the world but also on the sheep industry in the country has caused severe economic losses, hinder the development of sheep industry in China one of the main diseases, according to preliminary statistics, the disease each year to China caused a direct economic loss about $40,00,000,000 (Zhang *et al*., 2007b).

The pathogen and serum epidemiological survey of literature shows that different countries and regions in the prevalence of a Mycoplasma species flock, there are differences (Gao *et al*., 2007; Hernandez *et al*., 2006; Parham *et al*., 2006). In the country, caused by mycoplasma pneumonia of goats is widely popular, according to reports in the literature, the provincial epidemic strains vary in different areas including Mycoplasma, *Mycoplasma mycoides* subspecies, *Mycoplasma mycoides* subspecies and *Mycoplasma pneumonia* in goats subspecies but the same flock often appears in several Mycoplasma mixed infection, complicate the disease is difficult to disease prevention and control.

The county of Chongqing in recent years to develop aquaculture of goat from Shandong, Jiangsu, Sichuan, imported a large number of sheep due to long distance transport, raise the level of management and regional differences and other factors, after introduction, often cough, panting and sheep nasal fluid such as respiratory symptoms, identified by PCR, determine which is composed of filamentous the infection caused by *Mycoplasma mycoides* subspecies.

MATERIALS AND METHODS

Reagent and positive DNA preparation: Cell DNA Extraction Kit purchased from Qiagen; Marker was purchased from Takara company. Test strains positive for *Mycoplasma mycoides* subspecies (DNA Y-goat) genomic DNA was a gift from Yue-hua professor of Southwest University.

Animal, tissues and crude DNA preparation: Pleural effusion and lung tissue of a fatality sheep were harvested by aseptically removing them and immediately placing them in 1.5 L labeled snap-cap tubes, refrigerated transport to the laboratory. Crude DNA was obtained by boiling method: approximately 50 mg of tissue was homogenized in 500 μL of 1% Sodium Dodecyl Sulfate (SDS) in 100 mM Tris-HCl (pH 8.0), boiled for 10 min and centrifuged at 10,000 g for 5 min. The supernatant was transferred to a new tube and used immediately.

Design of the PCR primers: A set of specific primers was designed by targeting the sequence of *Mycoplasma mycoides* subsp. capri LC strain 285F08 16S ribosomal *RNA* gene (GenBank Accession No. GQ409972.1) using Primer 5.0. The nucleotide sequences of the primers are shown in Table 1.

Table 1: The primers used for PCR

Primers	Sequence (5-3')	pos	Length (bp)
MF	GCAAGTCGAACGGAGGTGCT	48-68	696
MR	TGCCTCAGCGTCAATAACAAGC	726-744	-

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PCR detection: PCR conditions were as Initial denaturation at 94°C for 30 sec and 30 cycles of PCR with 53°C for 30 sec and 72°C for 50 sec and extension at 72°C for 5 min.

RESULTS AND DISCUSSIONS

The PCR assay successfully amplified the target sequence of the *Mycoplasma mycoides* subsp. *Capri* LC strain 285F08 16S ribosomal *RNA* gene as observed by agarose gel electrophoresis. Imaging was completed with 20 g L^{-1} agarose-TBE gel electrophoresis; DL-2000 Marker was regarded as the reference object of PCR products (Fig. 1).

Use of Mycoplasma mycoides subspecies specific primers of MF and MR1 on the extraction of culture DNA was amplified by PCR, electrophoresis observed clear a specific objective strap, products for the 696 bp (Fig. 1). M marker, Y-goat for 1-4 laboratory strains, the same strain of different culture media culture.

The current diagnoses methods of mycoplasma pneumonia of sheep are the pathogen isolation and identification of Mycoplasma *in vitro* but the cost is high, culture conditions is demanding and the growth is slow, it cannot satisfy the needs of rapid diagnosis. PCR technology was widely applied in recent years in sheep mycoplasmal pneumonia diagnosis and monitoring because of its simple, rapid, specific and sensitive. In 1996, Hotzel established a PCR method for rapid and specific detection of Mycoplasma mycoides group which can identified of *Mycoplasma mycoides* family of 6 sub-populations (Hotzelh *et al*., 1996). Sophie *et al*. (2008) has established a PCR analysis method for specific and rapid detection of *Mycoplasma mycoides* subspecies type SC and goat *Mycoplasma pneumoniae* subspecies. In 2010, the application of *Mycoplasma mycoides* with *Mycoplasma ovipneumoniae* specificity primer on collected clinical nasal swabs and lung tissue were detected, *Mycoplasma ovipneumoniae* goat is one of the major causes of mycoplasma pneumonia (Yang *et al*., 2010).

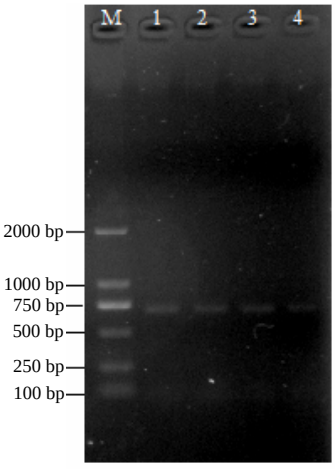

Fig. 1: Result of the PCR ; Lane M, DL-2000 DNA marker; Lanes 1): Y-goat; 2-4): Caprine *Mycoplasma pneumonia*

CONCLUSION

The clinical isolates of *Mycoplasma pneumoniae* by specific PCR for the identification of *Mycoplasma mycoides* subspecies which Cheng Guangsheng and in the Chongqing area isolated from *Mycoplasma mycoides* subspecies are different but both belong to the *Mycoplasma mycoides* cluster member (Guang-Sheng and Hao-Jv, 2006). Chongqing region of goats contagious pleuropneumonia pathogen is relatively complex, at the same time clinical immunity failure phenomena also have occurrence; the separation results enriched region of Chongqing sheep contagious disease survey data in Chongqing area for the effective control of infectious pleuropneumonia vaccine development has added new material.

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