SHORT REPORT

# The first case of goat infection with *Mycoplasma conjunctivae* in Ningxia Hui Autonomous Region, China: A case report

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***Mycoplasma conjunctivae*** **belong to the** ***Mycoplasma*** **species of the** ***Mycoplasma*** **family and is the main pathogen that causes infectious keratoconjunctivitis in goats. Infectious keratoconjunctivitis (IKC), also known as red eye disease, is a highly contagious disease that is prevalent worldwide. In October 2022, a goat farm in Tongxin County, Ningxia Hui Autonomous Region, China began to show leukoplakia in the eyes of goats 3 days after the introduction of a batch of goats without isolation observation. The sick goat showed listlessness and poor appetite with body temperature up to 41°C. The eyes began to appear white spots, and its range gradually expanded, covering the entire eyeball. Among the 200 introduced goats, 40 animals were affected with an incidence rate of 20%. Ocular secretions from 15 sick goats were collected, and DNAs were extracted and amplified by polymerase chain reaction using specific primers. The amplified products were then sequenced, and phylogenetic trees were constructed. The phylogenetic tree showed that Mco 01, Mco 02, Mco 03, Mco 04, Mco 05, Mco 06, Mco 07, Mco 08, Mco 09, Mco 10, Mco 11, Mco 12, Mco 13, Mco 14 and Mco 15 had the closest genetic distance with the reported** ***M. conjunctivae*** **Goat 655 and M165/69 strains. The self-test support rate with Goat 655 was as high as 98. This study reported, for the first time, the identification of** ***M. conjunctivae*** **in the ocular secretions of goats from Ningxia Hui Autonomous Region. Under the stressful conditions of long-distance transportation, the health status of goats should be highly concerned, and timely prevention of** ***M. conjunctivae*** **disease could improve the survival rate of purchased goats.**

**Keywords:** *Mycoplasma conjunctivae*; isolation; identification; phylogenetic analysis.

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## Introduction

*Mycoplasma* is intermediate in size between viruses and bacteria and presents a high degree of pleomorphism with the vast majority being round [1]. Because it is the smallest prokaryote lacking a cell wall, it is insensitive to antibiotics such as penicillin and cephalosporins acting on the cell wall [2]. *Mycoplasma* has contact and infectivity, and its host range is wide, which can cause *Mycoplasma* synoviae disease, *Mycoplasma* gallisepticum disease, *Mycoplasma* bovis disease, *Mycoplasma* ovipneumoniae, and infectious keratoconjunctivitis (IKC) [3].

*Mycoplasma conjunctivae* belong to the *Mycoplasma* species of the *Mycoplasma* family and is the main pathogen that causes IKC in goats

[4, 5]. *M. conjunctivae* was first isolated from the eyes of diseased goats in Australia by Surman in 1968. Four years later, Barile *et al*. isolated it from the eyes of diseased goats in Maryland and named it as *M. conjunctivae* [6]. IKC can be transmitted through direct contact with eye and nasal secretions of infected animals or indirect contact with mosquitoes, flies, contaminated utensils, and feed [7]. In the early stages of the disease, the unilateral eye of the sick animal is infected. The affected eye is shy of light and tearing. The eyelids are semi-closed, and the eye droppings are increased accompanied by purulent secretions [8]. With the continuous development of the disease, to the late stage, the disease will affect the other eye, resulting in binocular infection. The corneas were red, swollen, congested, raised and turbid, gradually thickened. The milky white and the protrusions covered the entire eyeball in a cloud shape [9]. Due to the aggravation of the disease, the cloud-like substance fell off, ulcerated, and corneal perforation occurred, resulting in irreversible damage and permanent blindness [10]. IKC disease, also known as red eye disease, is a highly contagious disease that is prevalent worldwide. It has been reported in Switzerland [11], Finland [12], and Spain [13]. Different breeds of goats can be infected with the disease [7]. Once animals are infected with this disease, the minor ones have difficulty in feeding and inconvenient movement, resulting in fat loss, increasing weight loss, and low feed conversion rate, which seriously damage the interests of farmers. The severe ones are in worse condition because they cannot find food, cannot maintain the wild survival, and even fall off the cliff [14].

Despite the worldwide prevalence of this pathogen, research on *Mycoplasma conjunctivae* has significantly lagged compared to other important animal pathogenic *Mycoplasmas*. In this study, *Mycoplasma conjunctivae* in goats were isolated and identified, which provided not only the direct evidence for the diagnosis of IKC, but also important experimental data and theoretical basis for the pathogenesis and transmission of the disease, as well as the prevention and treatment programs for the local susceptible animals in Ningxia, China.

## Materials and methods

### Animal sample collection

In October 2023, a goat farm in Tongxin County, Ningxia Hui Autonomous Region, China began to show leukoplakia in the eyes of goats 3 days after the introduction of a batch of goats without isolation observation. The sick goat showed listlessness and poor appetite with its body temperature reaching 41°C. Among the 200 purchased goats, 40 animals were affected at an incidence rate of 20%. The eye secretions of 15 sick goats were collected to determine the cause of this disease. The procedures of this research followed the operational guidelines of the Ningxia Hui Autonomous Region, China and was approved by the Science & Technology Ethics Committee of Ningxia University (Yinchuan, Ningxia, China) (Approval No. NXU-22-102). All farms' owners who participated in this study were provided with verbal informed consent based on the long and close cooperative relationship between both sides.

### Pathogen determination

2 mL of eye swab suspension was taken and centrifuged at 12,000 rpm for 2 min followed by genomic DNA extraction using a bacterial genomic DNA extraction kit (Tiangen, Beijing, China). The specific polymerase chain reaction (PCR) primers were designed and synthesized by Bioengineering (Shanghai) Shanghai Co., Ltd. (Shanghai, China) as McoF1: 5'-GTA TCT TTA GAG TCC TCG TCT TTC AC-3' and McoR1: 5'-CAG CGT GCA GGA TGA AAT CCC TC-3'. The PCR reaction system consisted of 2 µL of DNA template (<1 µg), 1 µL of each 10 µM primer, 25 µL of 2× Taq Master Mix (Sangon , Shang Hai, China), 21 µL of $ddH_2O$ to a final volume of 50 µL. The PCR reaction was performed using Bio-Rad S1000 thermal cycler (Bio-Rad, Hercules, California, USA) with 94°C for 2 min followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 1 min, then 72°C for 8 min. The PCR amplification products

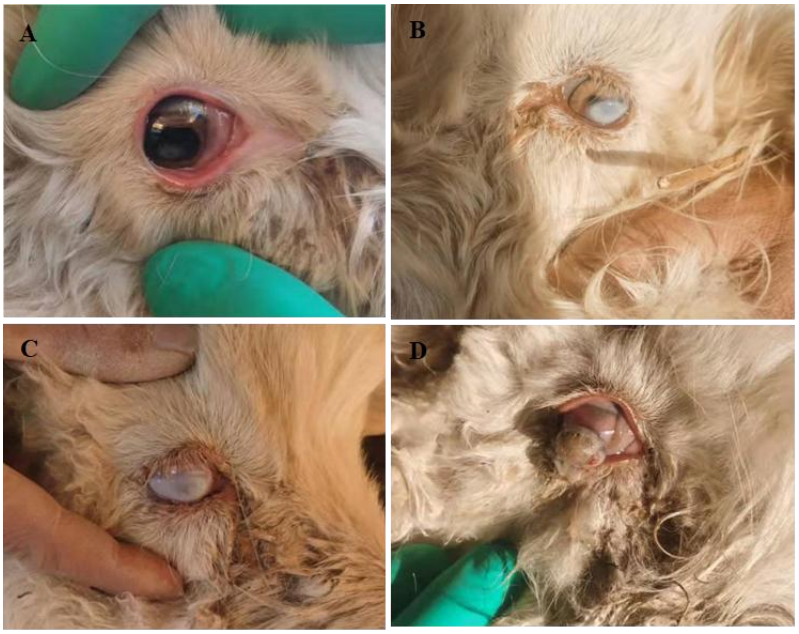


**Figure 1.** Pathological changes in the eyes of sick sheep.

were analyzed by 1% agarose gel electrophoresis. The PCR products were purified using gel recovery kit (Omega, FeiYang, Guangdong, China) and were sent to Bioengineering (Shanghai) Shanghai Co., Ltd. (Shanghai, China) for sequencing. The sequencing results were spliced using DNA star software (Lynnon Biosoft, San Ramon, CA, USA). The spliced sequences were compared to GenBank database using the BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The phylogenetic tree was constructed based on the spliced sequences using the Neighbor-joining (NJ) method in MEGA7.0 software (DNAStar, Madison, Wisconsin, USA).

## Results

### Clinical appearance

The eyes sick goats began to appear white spots, and its range gradually expanded, covering the entire eyeball (Figure 1). Through the diagnosis of clinical symptoms and autopsy results, the disease was preliminarily suspected as infectious keratoconjunctivitis caused by *Mycoplasma conjunctivae*.

### Construction of phylogenetic tree

The phylogenetic tree showed that *Mco* 01, *Mco* 02, *Mco* 03, *Mco* 04, *Mco* 05, *Mco* 06, *Mco* 07, *Mco* 08, *Mco* 09, *Mco* 10, *Mco* 11, *Mco* 12, *Mco* 13, *Mco* 14, and *Mco* 15 had the closest genetic distance with the reported *M. conjunctivae* Goat 655 and M165/69 strains, while the self-test support rate with Goat 655 was as high as 98 (Figure 2). It had a distant relationship with *Mycoplasma hyorhinis*, *Mycoplasma dispar*, and *Mycoplasma ovipneumoniae*. These isolates were further verified belonging to *M. conjunctivae*.

## Discussion

In this study, *M. conjunctivae* was first detected in goats from Ningxia Hui Autonomous Region. The sick goats showed clinical symptoms such as poor appetite, frequent blinking, corneal opacity, ulcers, and even blindness, which were highly consistent with the reports on the clinical symptoms of wild goats and antelope [15]. The symptoms such as frequent tears, increased eye feces, mild conjunctivitis, or corneal opacity in both eyes were observed in sick goats, which

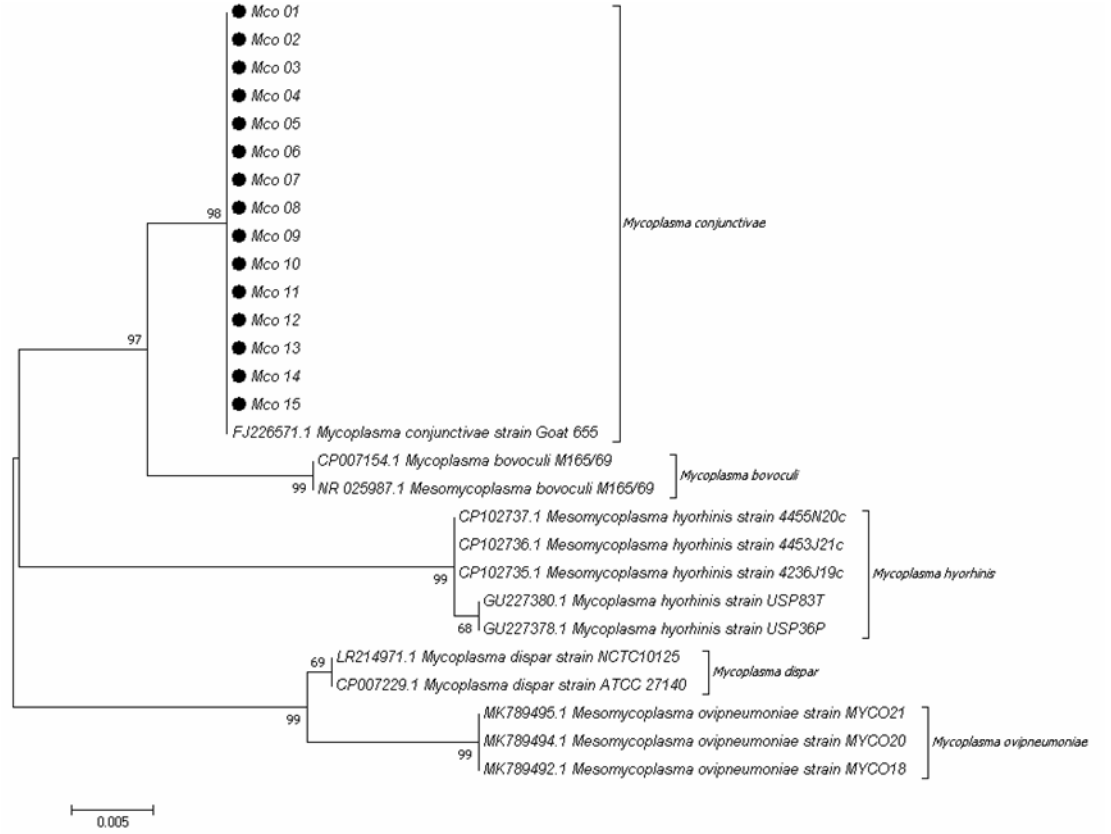


**Figure 2.** Phylogenetic tree constructed from *Mco* specific primer gene sequences.

were very similar to the typical clinical symptoms of small ruminants reported by Fernandez-Aguilar *et al*. [16]. After observing the sick goats, it was found that the clinical symptoms were mildly manifested as continuous tears, conjunctival congestion, and peripheral corneal edema. In severe cases, it was manifested as keratitis, corneal ulcer, and even corneal perforation, resulting in permanent blindness. Those manifestations were the same as the report of infectious corneal conjunctivitis in goat [17]. This study revealed that the clinical symptoms of captive goats infected with *M. conjunctivae* were the same as those of small ruminants such as wild goats, antelope, and goats.

*Mycoplasma* can escape the host's immune response in various ways, thus surviving in the host body [18]. Almost all these escape processes can cause changes in the structure and expression function of *mycoplasma* surface membrane proteins, so these mechanisms are essential for *mycoplasma* to adapt to the host and chronic colonization in the host [19, 20]. Once the *mycoplasma* is colonized in the body, it is difficult to completely remove it, which makes the body vulnerable to the invasion of other pathogens and easy to mix with other pathogenic microorganisms. Cases of mixed infection with pathogens of *Rickettsia* [21], *Mycoplasma agalactiae* [22], and *Chlamydia psittaci* [23] have been reported. Therefore, under the stress conditions of long-distance transportation, feed replacement, and environmental change, the health status of goats should be paid more attention, and the prevention of various diseases in time to improve the survival rate of purchased goats should be considered.

In China, long-distance transportation between livestock is an important factor in the spread of

animal diseases [24]. Due to the stress response to the long-distance transportation and the inadaptability of climatic conditions, the disease resistance of the animal body was reduced. Therefore, some conditional pathogenic bacteria could breed in large quantities, which led to the emergence of animal epidemics. In addition, the detection was not strictly strengthened before the introduction, and isolation observation was not implemented after the introduction. Because the introduction back was captive breeding, the breeding density was large, poor ventilation, and poor management. Bacteria and viruses would take the opportunity to enter the animal and cause disease. The genetic differences between animal individuals may also affect the occurrence, development, and prognosis of the disease [25]. This batch of goats had been introduced from another province. The related diseases were not detected at the time of introduction, and the isolation observation was not paid attention to after introduction. Therefore, to avoid similar situations, disease detection should be strengthened before introduction, and isolation should be carried out under the relevant requirements of veterinary rules and regulations after introduction. The quarantine period for large and medium-sized animals should be 45 days, while the quarantine period for small animals should be 30 days. During the quarantine period, a dry, clean, and well-ventilated environment should be provided. The anti-stress drugs should be given to animals to minimize the possibility of epidemic disease.

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