

## First identification of *Mycoplasma capricolum* subspecies *capripneumoniae* in goats in Iran

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Article Info	Abstract
<b>Article history:</b> Received: 03 June 2022 Accepted: 01 August 2022 Available online: 15 February 2023	<i>Mycoplasma capricolum</i> subspecies <i>capripneumoniae</i> (Mccp) is the etiological agent of caprine contagious pleuropneumonia (CCPP) disease. The CCPP is one of the most severe diseases of goats. A herd of 2,000 goats located in the countryside of Tehran city, Iran, was examined for the study in August 2021. In history taking, observation, inspection and clinical examination, high case fatality rate (46.00%) due to respiratory distress and high morbidity of pleuropneumonia (15.00%) syndrome were recorded. Accordingly, ten carcasses of goats were dissected. The epidemiological pattern of the disease, clinical examination findings and the signs of necropsy of dead patients were suspected to CCPP. Four lung samples of necropsied goats were sent to the laboratory for PCR test and in all of them, Mccp was detected and CCPP was also confirmed. The disease was controlled by two measures: (a) the whole herd was first treated with antibiotics (florfenicol and tylosin) and (b) then the Pulmovac-In vaccine was then administered. This study is the first documented report of CCPP occurrence caused by Mccp in Iran and shows the importance of availability of effective vaccines to control and prevention of CCPP.
<b>Keywords:</b> Goat Immunity <i>Mycoplasma capricolum</i> Pleuropneumonia Vaccine	

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### Introduction

*Mycoplasma capricolum* subspecies *capripneumoniae* (Mccp) is the etiological agent of Caprine contagious pleuropneumonia (CCPP) disease. The CCPP is one of the most severe diseases in goats.<sup>1</sup> The disease has three main characteristics: First, it affects the respiratory system. Second, it is highly contagious. Third, it has a high lethality.<sup>2</sup> In naive herds, disease morbidity and mortality rates can reach 100% and 80.00%, respectively.<sup>3</sup> The occurrence of CCPP in Iran has been reported to the Office International des Epizooties (OIE), however, to date, there has been no published report of this disease in Iran.<sup>4</sup>

The aim of this study was to describe clinical, gross pathological and histopathological aspects and control measures of first documented outbreak of CCPP occurrence in a goat herd in Iran.

### Case Description

A herd of 2,000 goats located in the countryside of Tehran city, Iran, was examined for the study in August 2021. Due to the economic problems, the herd was malnourished for two months and a high morbidity of respiratory distress (15.00%) and mortality due to lung infection (7.00%) were observed during the farm visit. There was a history of recent introduction without quarantine of 200 sheep and goats into the herd. In the study of the herd's history, it was observed that despite the antibacterial treatment of the involved animals, a significant proportion of patients with respiratory distress (46.00%) died within a period of three days after the onset of clinical signs and 15 patients with severe respiratory distress were daily added to the affected population. On average, seven goats died daily due to acute respiratory distress syndrome over a period of 20 days.

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Clinical examination of patients revealed painful tachypnea (average respiratory rate: 100 breaths per min), fever of 41.00 to 42.00 °C, tachycardia (average heart rate: 120 beats per min) and hearing friction sound in auscultation of both sides of chest. Ten carcasses of goats were dissected.

At necropsy, fibrin deposition on visceral pleura (Fig. 1A), pleural adhesions (Fig. 1B), lung consolidation (Fig. 1C), the presence of straw-colored fluid in the chest (Fig. 1C) and melting of lung tissue (Fig. 1D) were recorded. After necropsy, eight lung samples from four necropsied goats were sent to the laboratory for Mccp identification by PCR test and histopathology using Hematoxylin and Eosin (H&E) staining. Four samples were sent to the laboratory in ice, and four in 10.00% neutral buffered formalin.

Histopathologically, the lungs revealed lesions characteristic of suppurative bronchopneumonia. Blood vessels surrounding the bronchioles were engorged. A varying degree of hemorrhage was also observed (Figs. 2A and 2B). The bronchi, bronchioles and alveolar lumens were filled with inflammatory exudates comprising predominantly of neutrophils and a few mononuclear cells such as lymphocytes and plasma cells. There was epithelial hyperplasia of some bronchioles (Figs. 2C and 2D).

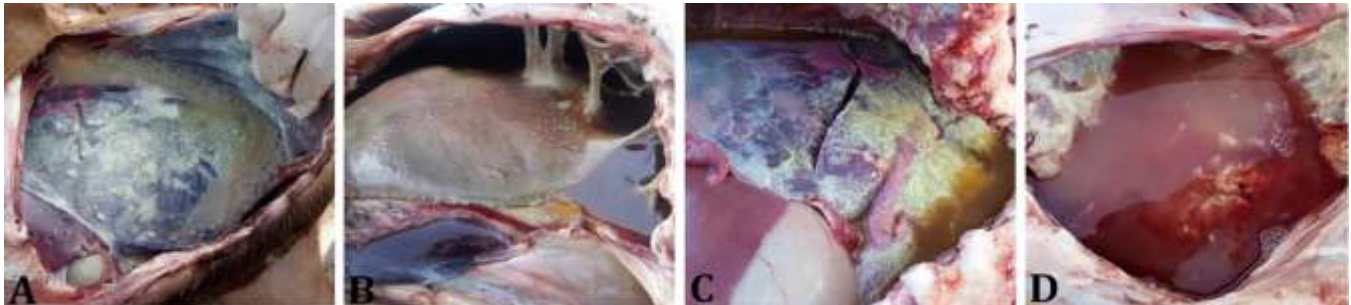
For PCR, DNA was extracted from the samples using the DNA extraction kit (Sinaclon, Tehran, Iran). The PCR test was performed according to the approach suggested by the OIE.<sup>5,6</sup>

As shown in Figure 3, the amplified part of the *arcD* gene with specific primers (F: 5'-ATCATTTTAAATCCCTCAAG-3',

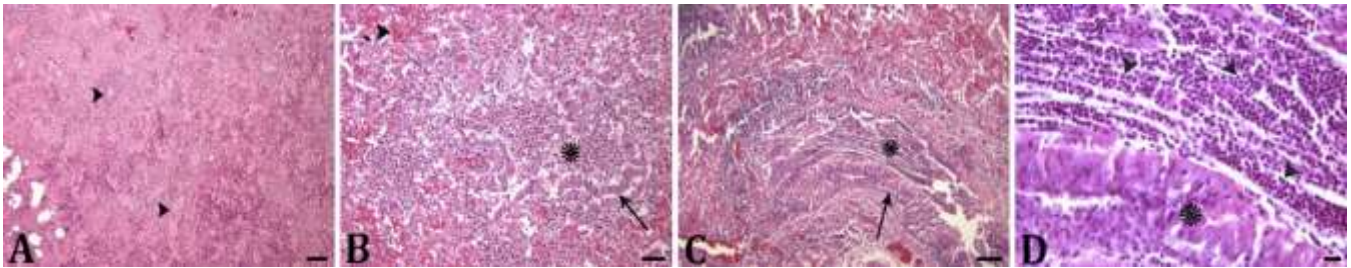
R: 5'-TACTATGAGTAATTATAATATATGCAA-3') showed the expected product size of 316 bp at agar gel electrophoresis indicating the presence of bacterial DNA of Mccp in the suspected lung samples.

Treatment and control were done in 3 steps. In the first step, the whole herd was treated with florfenicol (40.00 mg kg<sup>-1</sup>, SC, single dose; Razak, Tehran, Iran) and tylosin (10.00 mg kg<sup>-1</sup>, IM, every 24 hr for three days; Maymo, Vilamalla, Spain). After performing this step, the incidence of respiratory distress was stopped. In the second step, after the end of antibiotic therapy, the livestock storage area and all equipment were disinfected completely, and then, the whole herd was divided into two groups of vaccine (800 goats) and control (1,050 goats).

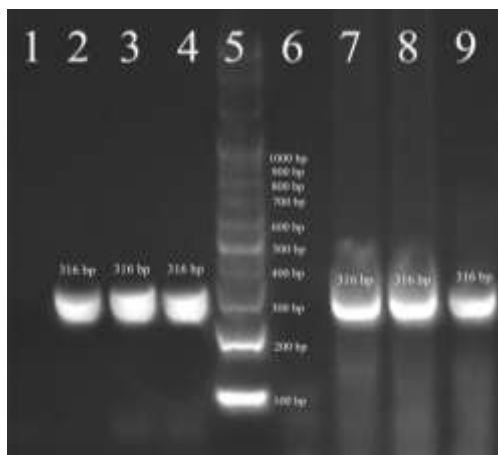
In the vaccine group, 1.00 mL (1 dose) of Pulmovac-In killed monovalent vaccine (containing a minimum of 0.15 mg mL<sup>-1</sup> of Mccp F<sub>38</sub> strain suspended in saponin as an adjuvant, Vetel Animal Health, Adiyaman, Turkey) was injected subcutaneously in the prescapular region. In the control group, no vaccine was prescribed. In the control group, 14 days after the end of antibiotic therapy, respiratory distress was recurred with the same epidemiological pattern as before. In the vaccine group, only two new disease cases were observed after two months post vaccination. In the third step, one month after recurrence of respiratory distress in the control group, the Pulmovac-In vaccine was obtained and its administration stopped respiratory distress in the control group. Delays in vaccine administration in the control group were due to difficulty in obtaining the vaccine.



**Fig. 1.** A) Fibrin deposition on visceral pleura; B) Pleural adhesion; C) Presence of straw-colored fluid in the chest; and D) Melting of lung tissue.



**Fig. 2.** Histopathological findings of Mccp in the lung tissue using H&E staining. A) Severe suppurative bronchopneumonia, affected bronchioles (arrowheads), (bar = 200 µm); B) and C) High magnification of bronchiole (arrows) and suppurative exudation (asterisks), hemorrhage (arrowhead), (bars = 50.00 µm, and 100 µm, respectively). D) Note the large numbers of neutrophils (arrowheads) in the bronchiolar lumen and epithelial hyperplasia (asterisk) of bronchiole, (bar = 20.00 µm).



**Fig. 3.** Polymerase chain reaction results of *arcD* gene amplification. Lanes 1, and 6: Negative control, Lanes 2, and 7: Positive control, Lanes 3, 4, 8, and 9: Lung tissue samples, Lane 5: DNA ladder (Sinaclon).

## Discussion

There is an OIE report of the CCPP occurrence in Iran and this report states that between 2006 and 2007, 478 outbreaks of CCPP occurred in Iran and 16,000 goats were affected.<sup>4</sup> Although there is an OIE report, to date, there have been no documented reports of CCPP occurrence in Iran. The first and last published attempt to find Mccp and CCPP in Iran was made by Namazi *et al.* but it was unsuccessful.<sup>5</sup>

The CCPP is one of the most dreaded disease of domestic and wild goats.<sup>4</sup> The spread rate of this Mycoplasma disease in the herd is unbelievably high, and most of the affected goats will die.<sup>7</sup>

During various studies, it has been shown that CCPP has an occurrence with a seasonal pattern and its occurrence is related to cold and heat stresses.<sup>8</sup> In this study, the occurrence of the disease coincided with heat stress. It has been determined that transportation stress and malnutrition are correlated with the incidence of CCPP<sup>9</sup> and in this study there was a history of two months of malnutrition in the herd.

In studies conducted in different countries, Mccp seroprevalence has been reported between 3.00 - 64.00%,<sup>8</sup> however, there are no published studies of this seroprevalence in Iran. Studies have shown that the morbidity rate of CCPP is 80.00 - 100% in non-endemic areas and 30.00 - 40.00% in endemic areas.<sup>8,10</sup> In current study, the morbidity rate was 15.00%, however, the author believed that if control measures were delayed this rate could have been reached 80.00 - 100%. The case fatality rate of CCPP has been stated in studies between 60.00 - 100%,<sup>1</sup> and in this study, it was 46.00%. This reduction in the case fatality rate could be due to the strong case-detection system and the constant presence and careful monitoring by the veterinarian in the farm.

Clinical signs and gross pathological findings observed in this study were similar to other studies.<sup>8,11</sup> The histopathological findings in this study were specific because in this study neutrophils and in other studies macrophages were the dominant cells within the alveoli.<sup>11</sup> In addition, perialveolar fibrosis<sup>8</sup> was not observed in this study. It seems that the authors' method of sampling the lung tissue was the cause of this difference, as the authors sampled the lung melting sections for histopathology.

It seems that tylosin is the antibiotic choice in the treatment of CCPP<sup>8</sup> and in addition, oxytetracycline, enrofloxacin, florfenicol, tulathromycin, and tiamulin are also effective in the treatment of the disease.<sup>12</sup> To date, vaccination with an inactivated whole microorganism in the vaccine (0.15 mg per goat of Mccp) has been the most effective tool for control and prevention of CCPP and provides clinical protection at a rate of 95.00%, however, the cost of Mccp culture to make the vaccine is very high.<sup>8,13</sup> In current study, Pulmovac-In vaccine was used. This study showed that, the Pulmovac-In vaccine was successful in controlling the disease.

In this study, Mccp and CCPP were identified and reported for the first time in Iran. In addition, it was found that the Pulmovac-In vaccine was effective in controlling CCPP.

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## Conflict of interest

The authors declare no conflict of interest.

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