



Research Article

Isolation and Characterization of *Mycoplasma capricolum* subsp. *capripneumoniae* from Sheep and Goat Pneumonic Lungs

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Keywords: Sheep; goat; *Mycoplasma capricolum* subsp. *Capripneumoniae*; PCR; Contagious caprine pleuropneumonia.

Abstract

Mycoplasma capricolum subsp. *Capripneumoniae* (*Mccp*) is the causative agent of contagious caprine pleuropneumonia (CCPP). In many countries CCPP is diagnosed by clinical signs and serological methods only. *Mccp* is known to be difficult to culture in the laboratories due to the fastidious nature of the organism. In the Sudan, there is need for further studies on sheep and goat pneumonia. The aim of this study was to determine the frequency of *Mccp* in ovine and caprine pneumonic lungs and to study the histopathology of the affected lungs. Tissue samples were obtained from condemned lungs of slaughtered sheep and goats and from clinically ill goats in Omdurman, Khartoum State, Sudan. Bacteriological culture and PCR methods were used to detect *Mccp*. Histopathological evaluation was also performed. *Mccp* DNA was identified in 4 goat and one sheep sample using PCR, while only one of them-goat lung sample- was identified using bacteriologic culture lung samples. None of the detected microscopic lung lesion could be considered specific for *Mccp* infection. This study documents for the first time, the presence of *Mycoplasma capricolum* subsp. *capripneumoniae* (*Mccp*) DNA in pneumonic sheep lung in Sudan suggesting that sheep may act as potential carriers of CCPP and/or clinically affected animal species. This highlights the need for further investigation into the epidemiological role of sheep in the transmission of CCPP. Although PCR is more costly than traditional bacteriological culture, it proves to be a valuable tool for CCPP surveillance due to its higher sensitivity and specificity.

Introduction

Mycoplasma capricolum subsp. *capripneumoniae* (*Mccp*) belongs to the so-called *Mycoplasma mycoides* cluster, which is a group of mycoplasmas of particular importance in veterinary medicine (Zaki, 2001). *Mccp* is the cause of contagious caprine pleuropneumonia (CCPP), a severe

OIEa-listed infectious disease of goats, it is of major economic importance in Africa, Asia, and the Middle East (El-Deeb et al., 2017; Abd-Elrahman et al., 2020; Ahmad et al., 2021). Clinical disease and seropositivity have been reported in sheep in contact with affected goats (OIE, 2008).

Wild ruminants, such as the wild goat, have also been shown to be infected (Ostrowski *et al.*, 2011).

In Sudan, CCPP is known as Abu Nini. The earliest reference to this disease was in the annual reports of the Sudan Veterinary Department (ARSVS, 1902). Goats with pleuro-pneumonia were first reported in what was then known as Kassala Province and subsequently from other provinces. In 1958, attempts to isolate the causative organism were largely unsuccessful (ARSVS, 1958). Karib (1953) succeeded in reproducing the lung lesions by spraying diluted lung fluid from an infected animal into the nostrils of three goats. Abdulla and Lindly (1967) achieved the primary isolation of *Mycoplasma* from a goat with pleuropneumonia, but the isolate could not be maintained. The first isolation and identification of the bacteria was achieved by Harbi *et al.*, (1981); and it was not isolated again- since that time- till 2006 when Sowsan Abbass (2006) isolated *Mccp* from a field case. All previous isolations were from goats showing typical signs of CCPP.

Mycoplasma capricolum subsp. *capripneumoniae* is known to be difficult to culture in laboratories (Freundt, 1983; Thiaucourt *et al.*, 1996), due to the fastidious nature of the organism (Nicholas, 2002; OIE, 2021). It has only been isolated in 13 out of 40 countries in which the disease has been reported. In many countries, CCPP is diagnosed by clinical signs and serological methods only (Mbyuzi *et al.*, 2014).

In Sudan, there is a need for studies on sheep and goat pneumonia with regard to etiology, breed, sex, age, predisposition, and the effect of season. The aim of this study was to determine the frequency of *Mccp* in ovine and caprine pneumonic lungs using conventional bacteriological methods and PCR.

Materials and Methods

Ten clinically ill goats suspected of contagious caprine pleuropneumonia (CCPP) and showing typical clinical signs were post-mortem examined. Lung tissue samples with gross pneumonic lesions were collected from two abattoirs in Omdurman city, Khartoum state, A total of 65 of ovine and 9 caprine lung samples were collected during dry summer and 65 sheep and 15 goat's lung samples were obtained during winter. Representative specimens were cut for bacteriological, molecular, and histopathological investigations. Portions of affected lung tissue were placed in sterile containers and transported on ice for culture and PCR analysis, while additional samples were fixed in 10% neutral buffered formalin for histopathology.

Bacteriological media was prepared as described by Zaki (2001), Sawsan Abbass (2006), Ajuwape *et al.* (2003) and Thiaucourt and Bölske (1996).

DNA was extracted from lung tissue samples using a DNA extraction kit (GF-DNA extraction kit) and proteinase K. following standard procedures. Polymerase chain reaction (PCR) assays were performed using Primers with a 316 base-pair size. Forward primer *Mccp*-spe-F: 5'-ATCATTTTAAATCCCTTCAAG-3' and Reverse primer *Mccp*-spe-R: 5'-TACTATGAGTAATTATAATATATGCAA-3', were used (Lorenzon *et al.*, 2008). The automated thermal cycler was set in accordance with the method described by Lorenzon *et al.* (2008) with modification of the last step. The initial step was denaturation, followed by an amplification step consisting of 40 cycles of denaturation, annealing and amplification. The final step consisted of one plateau at 72. Amplification products were separated by agarose gel electrophoresis, and gels were visualized under ultraviolet (UV) transillumination. DNA molecular weight marker (Vivantis, Malaysia) was included to estimate the size of the PCR amplicons. Bands of approximately 316 bp were considered positive for the presence of *Mccp* genomic DNA.

Formalin-fixed lung tissues were trimmed, dehydrated, cleared, and embedded in paraffin wax using an automatic tissue processor. Sections of 4–6 µm thickness were prepared and stained with hematoxylin and eosin (H&E) according to the method described by Bancroft and Stevens (1990). Stained sections were examined microscopically for characteristic pathological changes associated with CCPP.

Results

From the 130 ovine and 34 caprine samples that were cultured in *Mycoplasma* liquid and solid media, two samples collected during winter were found to be positive for growth. The growth of *Mycoplasma* was primarily indicated by colour change and/or appearance of follicular material. The turbidity of the Hayflick's liquid media that developed after a week of incubation was regarded as primary sign of growth. Colony morphology tests revealed two colonies, one of them (colony A) was relatively larger than the other (Colony B). The colonies were of fried egg appearance in penicillin and in penicillin-free modified Hayflick's solid media when tested for reversion of the L-form bacteria, there was no bacterial reversion in the two isolated colonies subcultured in the Penicillin free media. The two colonies did not ferment glucose, but both were positive for digitonin growth inhibition and tetrazolium reduction test. The samples from the sacrificed goats suspected to have CCPP revealed no *Mccp* growth.

When the two colonies were tested for *Mccp* genomic DNA using PCR, colony B showed a faint positive band (Fig. 1) whereas colony A was found to be negative for *Mccp* DNA.

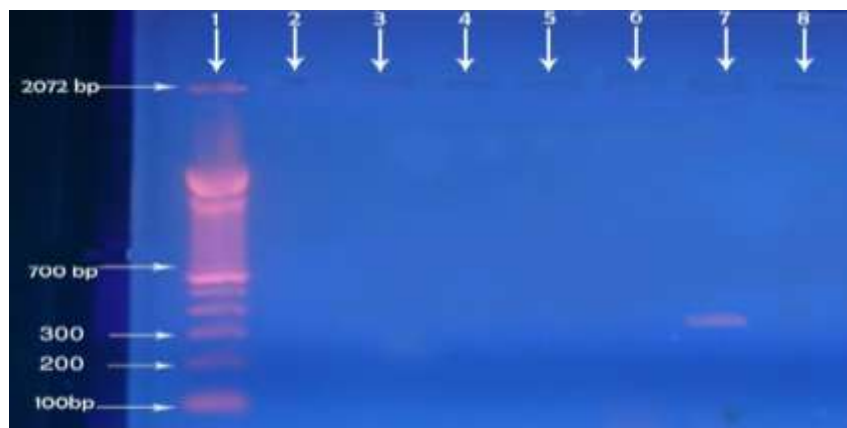


Fig. 1: Electrophoresis pattern of amplified *Mccp* DNA extracted from goat's lungs using PCR. 7 = Product of DNA amplification of 316 bp

Sheep and goat samples that revealed negative results for *Mccp* using the conventional cultural and biochemical methods were then tested for genomic DNA of *Mccp* using PCR. Four samples collected in winter were found to be positive, one of them was from a sheep.

the lungs condemned at meat inspection showed consolidation with reddish to greyish dull areas and thickened pleura with adhesion. Lungs of sacrificed goats were enlarged and heavy and showed similar lesions to those observed in the condemned lungs. These were seen in both left and right lungs. No pleural fluid was seen. Pleural adhesion was not prominent.

The lung sections of the five positive samples for *Mccp* DNA, showed marked alveolar collapse and presence of mononuclear cells and RBCs in the alveoli (Fig. 2; Fig. 3). Air passages showed necrotic degenerative changes of epithelial cells and presence of mononuclear inflammatory cells and exfoliated epithelium in the lumen (Fig. 4). Some sections showed severe congestion of alveolar wall capillaries.

Atelectasis and emphysema were detected in three lung sections with fibrin exudation in the alveoli of two of these sections (Fig. 5). Perivascular fibroplasia (Fig. 6) and hyperplasia of lymphoid nodules around an air passage (Fig. 7) were also detected.

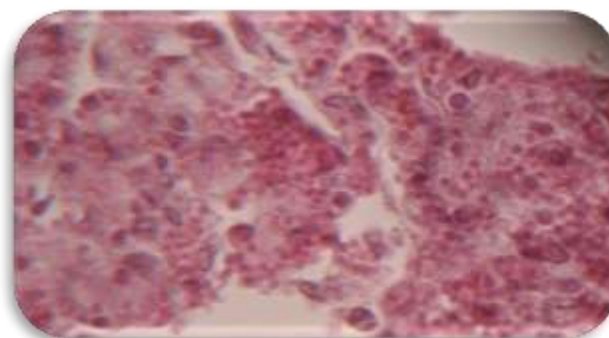


Fig. 3: Goat's lung, compact collapse with clumps of inflammatory cells and RBCs (H & E. X400).

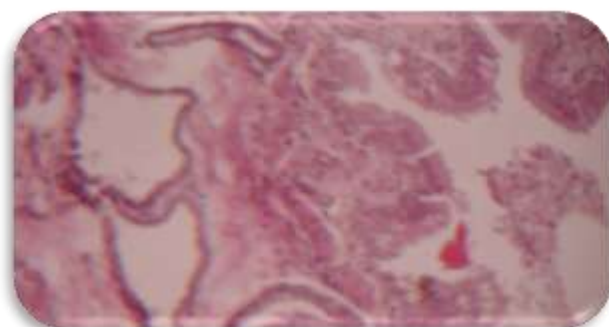


Fig. 4: Goat's lung, air passages showing degeneration of the epithelial cells and presence of detached epithelial and inflammatory cells inside (H & E, X400).

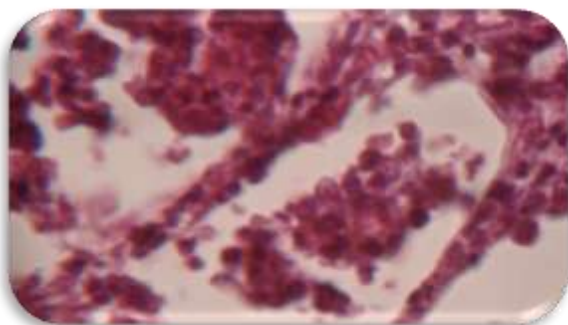


Fig. 2: Goat's lung. Showing infiltration of inflammatory cells composed of mononuclear cells inside the alveoli (H & E X400).

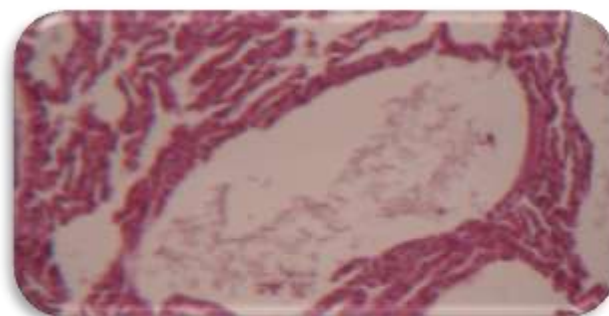


Fig. 5: Goat's lung. showing atelectasis and fibrin exudation inside the alveoli (H&E. X400).

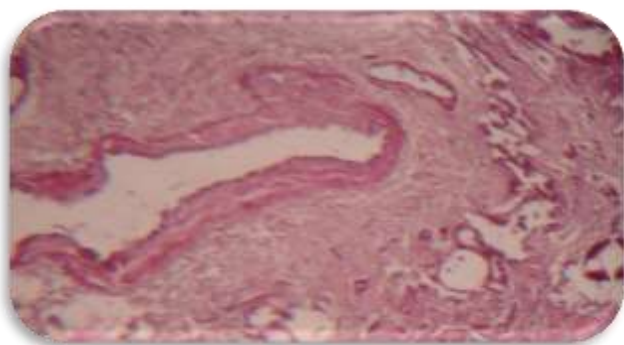


Fig. 6: Goat's lung showing perivascular fibroplasia (H & E, X100).

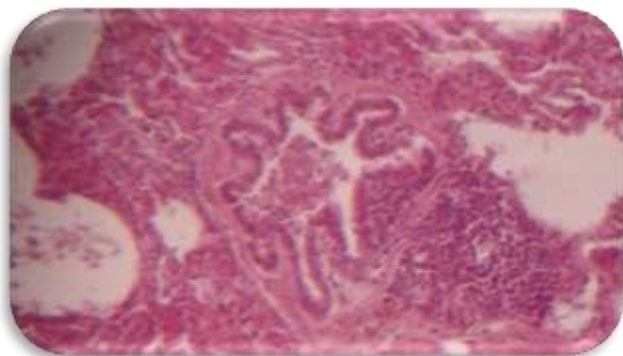


Fig. 7: Goat's lung showing nodular peribronchial lymphoid hyperplasia and intrabronchial cellular exudate (H & E, X200).

Discussion

Sheep and goats constitute the major livestock species in Sudan and are of considerable economic and cultural importance. They serve as a primary source of food and income for people living in poverty, refugees, displaced populations, and rural communities (, 2006; Kanani, 2009). Small ruminants play a vital socioeconomic role in the country and represent strategic resources for both domestic consumption and export (NSRS, 1990). In addition, they provide critical financial support for women in rural communities.

Respiratory diseases, particularly contagious caprine pleuropneumonia (CCPP), represent major constraints to efficient sheep and goat production in Sudan. These diseases threaten the livelihoods and stability of rural households, especially those of women who depend on small ruminant rearing to meet daily living costs and education expenses.

The present study investigated the occurrence of *Mycoplasma capricolum* subsp. *capripneumoniae* (*Mccp*) in pneumonic lungs of sheep and goats in Khartoum State, Sudan, using both conventional bacteriological techniques and PCR. Our bacteriological results showed very limited isolation of mycoplasmas, with only two colonies detected in winter samples, one of which (colony B) was confirmed as *Mccp* by PCR. These results agree with previous reports that *Mccp* is fastidious and extremely difficult to isolate

under routine laboratory conditions (Thiaucourt and Bolske, 1996; Nicholas and Churchward, 2012). The organism requires complex media, supplemented with horse serum and yeast extract, and often has a slow growth rate, making culture results inconsistent (OIE, 2021). The findings confirmed the presence of *Mccp* DNA in sheep and goats, despite the challenges of isolating the organism in culture. The detection of *Mccp* DNA by PCR in four additional samples (three goats and one sheep) highlights the higher sensitivity of molecular techniques over conventional culture methods. Interestingly, one sheep sample tested positive for *Mccp* DNA, supporting previous evidence that sheep can act as subclinical carriers or may develop disease when in contact with infected goats (Litamoi *et al.*, 1990; Wesonga *et al.*, 2004). Although goats remain the primary host of CCPP, the role of sheep in epidemiology should not be underestimated, especially in mixed grazing systems common in Sudan.

The gross and histopathological findings currently recorded are those of subacute pneumonia and bear resemblance to CCPP lesions. However, in typical cases of CCPP, the lesions are confined to the lung and pleura and are often unilateral but other bacteria may cause unilateral lung infections leading the limited isolation or identification of *Mccp* in such tissues which was high suspected.

In acute cases, affected lungs can be totally hepatized. There is often pleuritis pleural effusion (Kalinier and MacOwan, 1976; Rurangirwa *et al.*, 1981; Wesonga *et al.*, 1993). Microscopically fibrinous edema is commonly detected in intralobularly and the interlobular septa are seldom enlarged. However, no microscopic pathognomonic lung lesion could be considered specific for *Mccp* infection. Lesions like what has been described in this study can be seen in other pulmonary infections. In the present study, no *Mycoplasma* was detected in the pneumonic lung tissue collected during summer from slaughtered goats. Inability to collect pleural fluid could have been a factor that affected the frequency of isolation of *Mccp* from the condemned sheep and goat lungs. Recently, polymerase chain reaction-based tests have been described and shown to be specific and sensitive and can be applied directly to clinical materials (OIE, 2008). When the same lung tissue samples were tested for the presence of *Mccp* DNA using conventional PCR, they revealed five positive samples, including only one of the above two microbiologically detected samples. Four of the five positive samples for *Mccp* DNA (one of them is an ovine sample) were collected during winter. This suggests the low temperature and crowding of animals in winter predispose them to *Mccp* infection.

The role of *Mccp* in sheep respiratory tract infections warrants further investigations. Reports from Kenya (Litamoi *et al.*, 1990_b) and Uganda (Bölske *et al.*, 1995) describe the isolation of *Mccp* from sheep in contact with

goat herds affected with CCPP. In Egypt, CCPP was detected in 32.5% of the examined sheep with an 8% fatality. Antibodies against *Mccp* have also been found in sheep (Mbyuzi *et al.*, 2014). However, one question could be raised as to whether sheep could be carriers for the disease.

In the present study, four out of 34 examined goat lungs (11.8%) were found to be infected. As reported in previous investigations, the isolation of *Mycoplasma capricolum* subsp. *capripneumoniae* (*Mccp*) by conventional bacteriological methods remains challenging due to the fastidious nature of the organism, which influences the low frequency of isolation (20%) compared to PCR. However, PCR results may also underestimate the actual magnitude of the disease in Sudan, as they are affected by the quality of DNA extracted from formalin-preserved samples. The faint positive band detected in one isolate in the current study could be explained by partial DNA degradation during preservation, storage, and processing, an issue previously highlighted by Lorenzon *et al.* (2002).

In the present study, four goat lungs out of 34 goat lungs-constituting 11.8%- were found to be infected with *Mccp*, as mentioned in previous studies, isolation of *Mccp* using conventional bacteriological methods is difficult and it may affect the frequency of isolation of *Mccp* which was found to be 20% of the detected using PCR as it is affected by the fastidious nature of the organism. PCR results also may underestimate the magnitude of the disease in the Sudan as it is affected by the quality of DNA of the formalin preserved samples. The faint positive band observed in one isolate in the current study may be attributed to partial degradation during preservation, storage and processing, a limitation that has also been noted elsewhere (Lorenzon *et al.*, 2002).

The gross lesions observed in slaughtered and sacrificed animals-consolidation, pleural thickening, and adhesions-are characteristic of CCPP (Rurangirwa *et al.*, 1987; Thiaucourt *et al.*, 1996). The histopathological findings of alveolar collapse, fibrin exudation, lymphoid hyperplasia, and perivascular fibroplasia are consistent with the severe inflammatory response and fibrinous pleuropneumonia described in experimental and field cases of CCPP (Abu Elzein *et al.*, 1999; OIE_b, 2021).

The seasonal variation observed in this study, with the positive cases detected only in winter, may reflect environmental stressors and management practices that increase susceptibility to respiratory infections. Cold weather, overcrowding, and poor ventilation are known to exacerbate the spread and severity of respiratory pathogens in small ruminants (Radostits *et al.*, 2007). Further studies with larger sample sizes are needed to confirm the influence of seasons on CCPP in Sudan.

In conclusion, this study provides evidence of *Mccp* in both goats and sheep in Khartoum State, Sudan, confirmed by PCR. Up to our knowledge, it is the first detection of *Mccp* in pneumonic sheep lung. The role of sheep in maintaining the disease should not be neglected. The results also highlight the diagnostic challenges posed by the organism and emphasize the importance of molecular methods for reliable detection of such a disease of significant economic and animal health impact in Sudan.

Statement of Animal Rights

We undersign, certificate that the procedures and the experiments we've done respect the ethical standards in the Helsinki Declaration of 1975 as well as the national laws in Sudan.

Conflict of Interest Statement

The author(s) declare(s) that there is(are) no conflict(s) of interest.

Author's Contribution

Hind E. Osman designed the research plan, performed experimental works & collected the required data. Hind E. Osman, Ahmed A. Gameel & Suliman M. El Sanousi analysed the data; Hind E. Osman prepared the manuscript. All authors critically revised, and finalized the manuscript. Final form of manuscript was approved by all authors.

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