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Molecular characterization and pathogenicity of *Mycoplasma capricolum subsp. capricpolum* from goats in Morocco

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Abstract

Mycoplasma capricolum subsp. capricolum (Mcc) is an important etiological agent of contagious agalactia (CA). CA affects small ruminants and is characterized by inducing mastitis, arthritis, kerato-conjunctivitis and respiratory symptoms. The aim of this study was to isolate and characterize Mcc from Moroccan goats with contagious agalactia. A total of 300 Alpine goats were monitored. Serology analysis, molecular identification, and isolation of Mcc were realized from suspected goats. An experimental study was conducted for isolated Mcc to determine their pathogenicity. Thus, clinical observation showed that respiratory symptoms were predominant in young animals, and other symptoms, such as mastitis, keratoconjunctivitis and lameness, were more frequently detected in adult goats. Of the 80 tested blood samples, 28 sera were seropositive for Mcc antibodies. Mcc was identified by polymerase chain reaction (PCR) in milk, lung tissue and synovial liquid samples. The isolation of Mcc was successful through bacterial culture from lung tissue. LppA gene sequence of this strain revealed 98.1% similarity with the reference strain (ATCC 27343), with 11 missense variants. Experimental infection resulted in severe and generalized CA disease in sheep and goats, confirming the high pathogenicity of the Moroccan Mcc isolate.

Keywords: Contagious agalactia, *Mcc*, *Goats*, *Sheep*, *Morocco*

Introduction

Mycoplasma are small and fastidious bacteria belonging to a group of *Mollicutes*. *Mycoplasma* are characterized by a minute genome size (0.58 to 1.35 kb) and are devoid of the cell wall (Brown et al., 2005). Thus far, *Mycoplasma* identification has been based on serological assays after cultivation. However, efforts have been directed toward replacing these assays with more rapid and accurate molecular approaches based on the detection of specific DNA sequences (Nicholas et al. 2008). *M. capricolum subsp. capricolum* (Mcc) could be identified using 16 sRNA polymerase chain reaction (PCR), restriction fragment-length p[olymorphism (RFLP) in rRNA genes and biochemical tests. However, these techniques may present cross-reactivity; members of the *M. mycoides* cluster are closely related genetically. PCR of *LppA* gene is recommended for the specific identification of Mcc (Monnerat et al. 1999).

In small ruminants, *Mycoplasma* is known to cause respiratory diseases, arthritis, ocular lesions, genital symptoms and mastitis. Mcc is one of the agents responsible for contagious agalactia syndrome (CA), together with *Mycoplasma agalactiae*, *Mycoplasma putrefaciens* and *Mycoplasma mycoides subsp. capillri* (Bergonier et al., 1997). Mcc causes sporadic outbreaks of caprine arthritis, polyarthritis, mastitis, keratoconjunctivitis, pneumonia, septicemia and vulvovaginitis with high morbidity and mortality rates (Pourbakhsh et al. 2015).

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Described for the first time in California as a virulent agent of CA in goat kids, the primary clinical sign was severe polyarthritis affecting nearly every diarthrodial joint (DaMassa et al. 1992). In France and Canary Islands, Mcc is the second most frequently isolated CA agent, and its presence has been associated with severe outbreaks (De la Fe et al. 2007). In Morocco, one study reported Mcc identification using biochemical and serological methods (Benkirane et al. 1993).

Recently, Mcc was also detected in a USA human patient displaying symptoms of septicemia (Heller et al. 2015).

In this study, investigations focused on suspected clinical cases of CA in a dairy group presenting mastitis, pneumonia and arthritis in young and adult animals. Mcc was isolated, sequenced and characterized for its pathogenicity in sheep and goats for the first time in North Africa.

Results

Case description

An alpine goat flock of approximately 300 head of dairy production, located in the center of Morocco, was suspected of having contagious agalactia. Clinical field observations showed a morbidity rate of approximately 19% in young goats and 5.7% in adult goats with mortality rates of 3.5% and 0.6%, respectively.

Young animals (10%) had respiratory symptoms, including cough, nasal discharges and dyspnea. However, six adult goats showed mastitis, two presented keratoconjunctivitis and one had lameness and arthritis. At postmortem examination, the lungs showed lesions of congestion, edema and hepatization, and hemorrhagic streaks in the trachea.

Mcc detection

Among samples collected from goats presenting symptoms of CA, two lung tissue, two milk and one synovial liquid samples were analyzed by conventional PCR and detected as positive for Mcc and negative for other mycoplasmas (*M. capricolum subsp capripneumoniae, M. mycoides subsp. capri, M. putrefaciens,* and *M. agalactie).* By real-time PCR, the cycle threshold (Ct) was lower in lung tissue (18.3) and in milk samples (19.0) (Table 1).

Bacterial isolation

Mycoplasma isolation was performed from the lung tissue of dead Alpine goats. From culture-positive plates, typical fried-egg bacterial colonies were observed in PPLO agar cultures after 5 d of incubation (Fig. 1). In PPLO broth, turbidity was observed after 48 h at 37 °C in a shaker incubator. A total of five isolates were collected and identified.

Sequencing and phylogenetic results

PCR amplification of *LppA* gene of the Mcc isolate showed the presence of a specific band of 1356 bp. Direct sequencing of the PCR products showed the presence of 11 missense variants when compared with *LppA* gene sequence of the Mcc American Type Culture Collection (ATCC) strain by ClustalW multiple alignment. The missense variants were D101N, S120F, K170R, A241T, P281S, T317A, G334H, H351Y, K408E, M413G and K481N. Using the Consurf server, we identified three amino acids (S120, K170 and K481) that were highly conserved and exposed, one amino acid (H351) that was highly conserved and buried and seven amino acids (D101, A241, P281, T317, G334, K408 and M413) that were highly variable and exposed. The sequence alignment by the basic local alignment search tool (BLAST) revealed an identity of 98.10% with *Mycoplasma capricolum subsp. capricolum* (ATCC 27343), followed by *Mycoplasma laechii* 99/014/6 (72.08%), *Mycoplasma feriruminatoris* G1650 (72%), *Mycoplasma mycoides* strain T1/44 GP (72.05%), *Mycoplasma mycoides subsp. capri* strain GM12 (71.89%) *Mycoplasma capricolum subsp. capripneumoniae* strain 033C1 (66.08%) and *Mycoplasma putrefaciens* strain NCTC10155 (63.33%). *LppA* gene sequence-based phylogenetic tree confirmed the close relationship between the isolate and ATCC strain, while *Mycoplasma*

Table 1 PCR-based identification of Mycoplasma species from samples of goats

N°	Source of samples	cPCR					qPCR (Ct value)
		Mcc[a]	Mccp[b]	Mmc[c]	M.put[d]	M. aga[e]	Mcc
1	Lung	+	-	-	-	-	18.3
2	Lung	+	-	-	-	-	38.4
3	Milk	+	-	-	-	-	19.0
4	Milk	+	-	-	-	-	26.8
5	Synovial liquid	+	-	-	-	-	32.4

qPCR Quantitative polymerase chain reaction, *cPCR* Gel-based polymerase chain reaction
[a]*Mycoplasma capricolum subsp. capricolum*, [b]*Mycoplasma capricolum subsp. capripneumoniae*, [c]*Mycoplasma mycoides subsp. capri*, [d]*Mycoplasma putrefaciens*, [e]*Mycoplasma agalactiae*, + positive, - negative

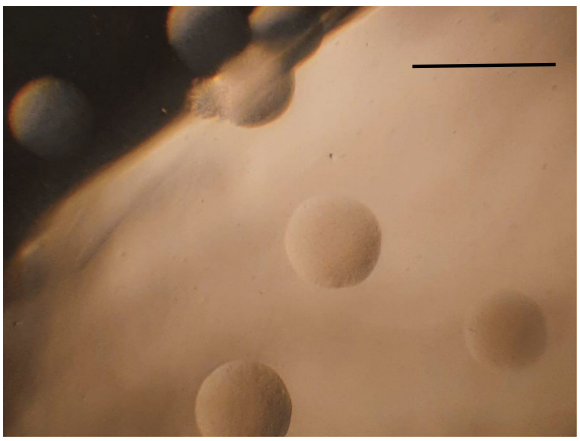

Fig. 1 Colonies of *Mycoplasma capricolum subsp. Capricolum* on PPLO agar with a characteristic fried egg appearance. bar means1 mm

laechii (99/0 14/6 and PG50) was phylogenetically the closest to *Mycoplasma capricolum subsp. Capricolum* (Figs. 2 and 3). Additionally, *LppA* gene sequence of Mcc MOR20 has been deposited previously in GenBank (MZ441164).

Antibody detection

Among 300 alpine goat samples, 80 sera from different age categories were tested. Twenty-eight animals were positive for specific antibodies against Mcc, with an overall prevalence of 35%. Adult goats presented the highest rate (18.75%), followed by goats at puberty and newborns with a rate of 6.25% (Table 2).

Clinical scoring and postmortem lesions

Following the challenge of animals with the Mcc MOR20, all sheep and goats developed respiratory symptoms, including nasal discharge and cough. Diarrhea was present mainly in goats (4/6) compared to sheep (1/6). Lameness was observed in young sheep and goats of G2 and G4 (6/6) and one adult local goat of G1 with a swollen and hot joint. Keratoconjunctivitis was observed in goat kids (3/3). The average clinical score was the highest in goat kids (18, 3), followed by lambs (16, 6), adult goats (12, 6) and sheep (11) (Table 3).

Mortality was important and occurred earlier in challenged lambs (3/3 at Day 8 post infection (pi)) than in goat kids (2/3 at Day 6-8 pi). Two sheep and one goat also died after the challenge between Days 12 and 17 pi (Table 3).

At autopsy, gross changes were confined to the lungs, joints and intestines. Lungs showed consolidation, pleural adhesion and a deposit of fibrin in the groups of adult animals (G1 and G3). Only one adult goat and one adult sheep resisted the challenge with no lesion at autopsy. In the groups of young small ruminants (G2 and G4), only consolidation and patchy areas were observed in the lungs. In animals presenting diarrhea and lameness, autopsy examination showed intestinal inflammation with enlarged mesenteric nodes, and fibrinopurulent exudate and hemorrhagic erosions of the cartilage were present in the articulation (Fig. 4).

Histopathological investigation

Animals infected with Mcc MOR20 showed bronchointerstitial pneumonia characterized by the coexistence of histological features of both bronchopneumonia and interstitial pneumonia. There was marked hyperplasia of the bronchi epithelium with a bronchi lumen containing an exudate of mucoid material mixed with polymorphous inflammatory cells, mainly neutrophils (Fig. 5a, hematoxylin and eosin (H&E) x20). The peribronchial and perivascular lymphoid nodules were hyperplasic. The alveolar space contained an inflammatory cell exudate, mainly neutrophils (Fig. 5b, H&E x20). In several areas, the interalveolar septa were markedly thickened due to massive mononuclear leukocyte infiltration in association with blood vessel congestion (Fig. 5c, H&E x40). Fibrinous pleurisy was also observed (Fig. 5d, H&E x20).

Discussion

In 2019, the World Health Organization (WHO) listed Contagious Agalactia for Animal Health among the most serious diseases of small ruminants caused by mycoplasma after contagious caprine pleuropneumonia (CCPP). However, available data are insufficient to evaluate the current prevalence and clinical severity throughout affected areas (Jaÿ and Tardy 2019). In Canary Islands, CA due to Mcc was first reported in 1992 and is still one of the major small ruminant health problems (G. Mogoş et al. 2021). Pourbakhsh et al. also detected Mcc in Iran in 2015 for the first time, while in Europe, CA was declared sporadic by Jaÿ and Tardy in 2019. In North Africa, where a small ruminant population is dominant, few data are available on CA prevalence and causative agents. Benkirane et al. (1993) isolated a few strains of mycoplasma from goat lung in Morocco that have been identified as Mcc based on biochemical tests.

The aim of this study was to genetically characterize and evaluate the pathogenicity of a new local strain of Mcc isolated from a goat dairy farm presenting clinical signs of CA in adult and young animals. Genetic analysis revealed that the isolate shares 97% similarity with the Mcc California strain, originating from goats, the only available strain of animal origin in GenBank (CP000123). The isolate presented 62% similarity with Mccp, which is in

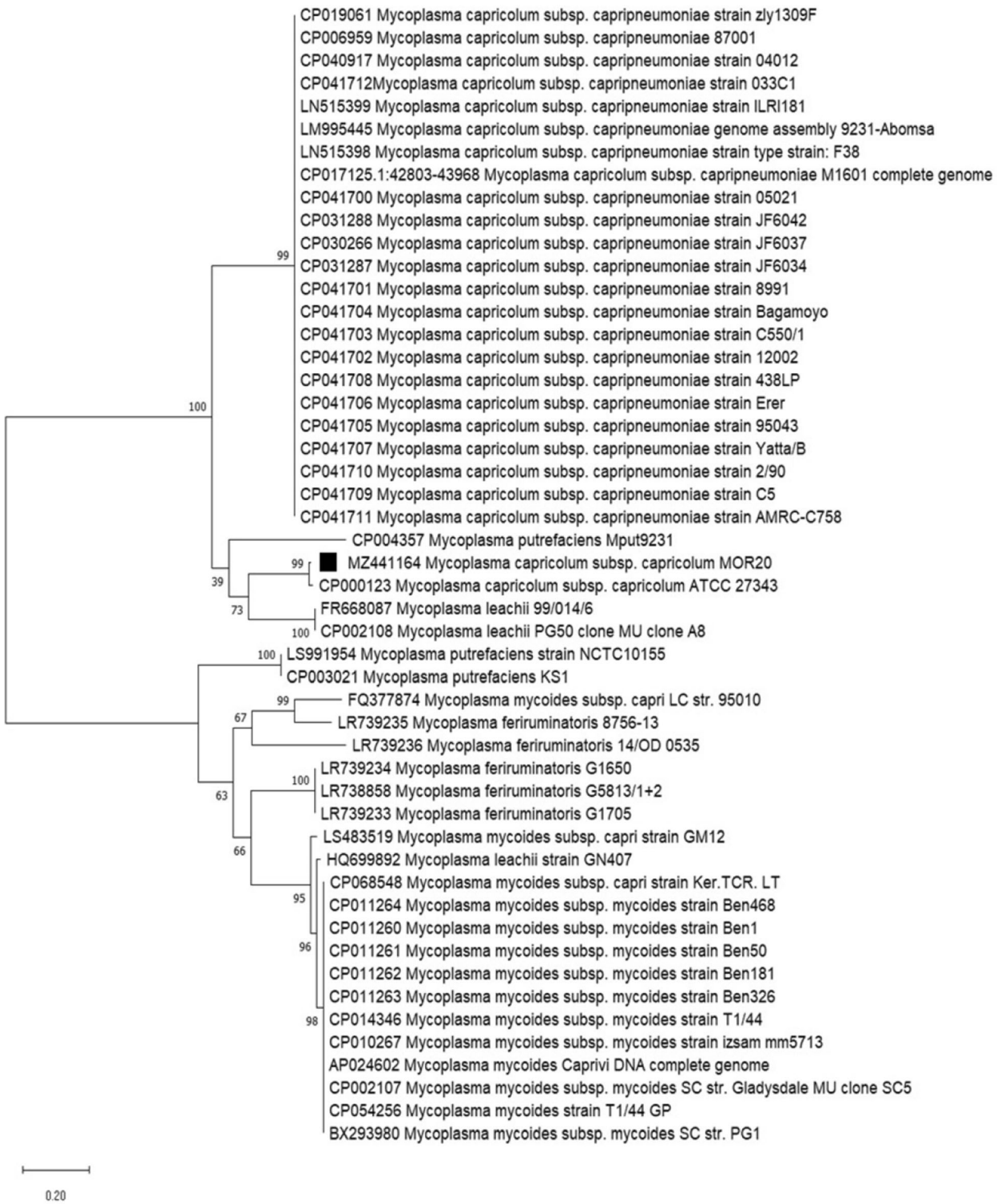

Fig. 2 Phylogenetic and molecular evolutionary analysis of the *LppA* gene (1356 bp) region conducted using Molecular Evolutionary Genetic Analysis Version 7.0 (MEGA7) with the Kimura two-parameter model and the Maximum Likelihood Statistical Method. The horizontal lines are proportional to the distance among sequences

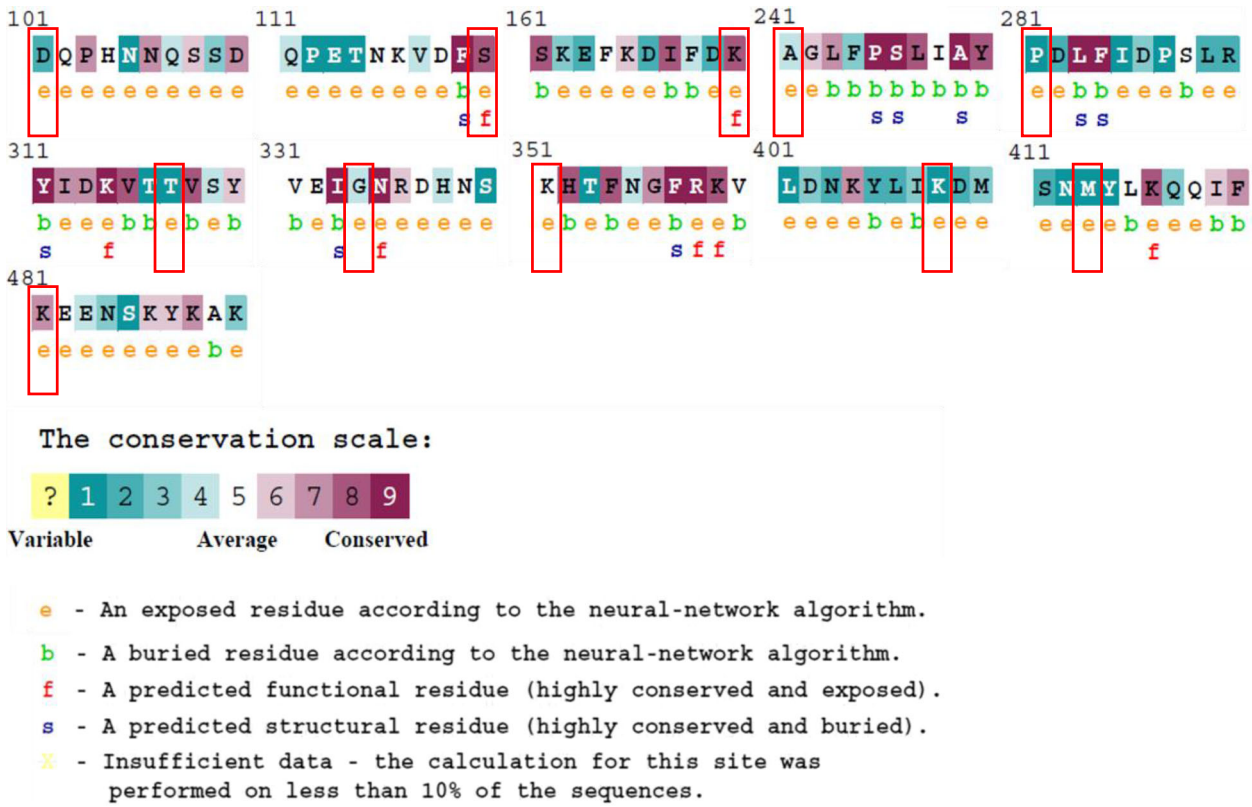

Fig. 3 Consurf profile of LppA protein of Mcc ATCC 27234. The red rectangle marks the position value of amino acids of interest

accordance with Monnerat et al. (1999), who stated that the LppA gene allows distinguishing between Mcc and Mccp, two members of the same species.

Clinically, three major signs have been observed in the affected goat flock: pneumonia in young and mastitis, kerato-conjunctivitis and arthritis in adults. All observed symptoms were exclusively due to Mcc, since no other mycoplasma was detected by PCR or culture in collected specimens from the sought animals. This finding, in accordance with a report in the Canary Islands, provides evidence that Mcc alone can induce CA with severe symptoms that can lead to mortality (De la Fe et al. 2007). In Iran, infection by Mcc induced only respiratory symptoms and lesions in young and adult animals; the difference from our results is probably linked to the strain virulence (Pourbakhsh et al. 2015).

Under normal field conditions, CA causes mortality only in young animals with acute pneumonia after being contaminated during milk sucking. Adult goats may recover from the disease or maintain a subacute infection such as mastitis and arthritis (Taoudi et al., 1987). Serology results in our study showed 35% herd seropositivity mainly in adults who have probably recovered from the clinical disease and in some newborns due to passive transmission of maternal antibodies.

Experimental infection with the MOR20 isolate produced a severe and generalized disease in sheep and goats, in both young individuals and adults. All 12 infected animals developed respiratory symptoms, and seven presented lameness and kerato-conjunctivitis and five diarrhea. Two goats and one sheep resisted

Table 2 Percentages of Mcc-positive goats

Category	Age of animals	No. of sera examinated	No. of positive animals	% of positive animals
1	≤1.5 month	20	5	6.25%
2	2-3 month	20	3	3.75%
3	9-12 month	20	5	6.25%
4	2-3 years	20	15	18.75%

Table 3 Results of clinical signs and anathomopathological lesions

Group	N°	Species	Breeds	Age	Clinical signs	Clinical score	Day mortality	Anathomopathological lesion						
								Thoracic lesion			Lymphadenitis		Intestinal inflammation	Synovitis
								Consolidation Lung	Pleural adhesions	Pleural fibrin deposits	Prescapular lymph node	Mesenteric node		
1	**467**	Goat	Local	12-18 months	Resp. Lam and ker. Dig.	22	14	+	+	+	+	+	+	+
	494	Goat	Local	12-18 months	Resp Dig	11	E	+	+	+	-	+	+	-
	496	Goat	Local	12-18 months	Resp.	5	E	-	-	-	-	-	-	-
2	**747**	Goat	Alpine	6-8 months	Resp. Lam and ker. Dig	13	E	-	-	-	-	-	-	-
	744	Goat	Alpine	6-8 months	Resp. Lam and ker.	19	8	+	+	+	-	-	-	+
	750	Goat	Alpine	6-8 months	Resp. Lam and ker. Dig.	23	6	+	+	+	+	-	-	+
3	**998**	Sheep	Local	12-18 months	Resp.	15	12	+	+	+	-	-	-	-
	562	Sheep	Local	12-18 months	Resp.	15	17	+	+	+	-	-	-	-
	524	Sheep	Local	12-18 months	Resp.	3	E	-	-	-	-	-	-	-
4	**394**	Sheep	Local	6-8 months	Resp. Lam and ker.	15	8	+	+	+	-	+	+	+
	168	Sheep	Local	6-8 months	Resp. Lam. Dig.	20	8	+	+	+	-	+	+	+
	267	Sheep	Local	6-8 months	Resp. Lam.	15	8	+	+	+	-	+	+	+

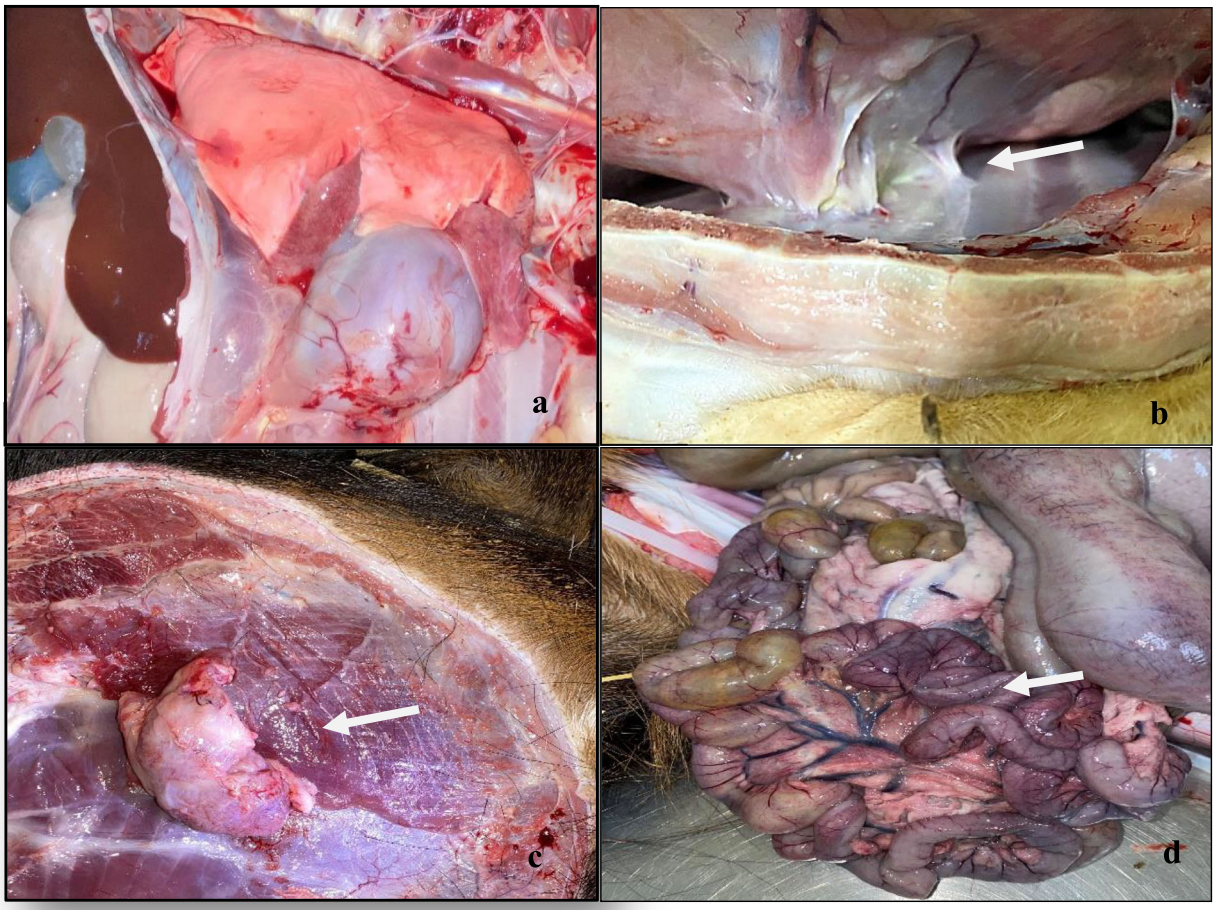

Fig. 4 Lesions following experimental infection of goats and sheep with *Mycoplasma capricolum susbp capricolum* isolate. **a** Lung consolidation. **b** Pleural adhesion and pleural fibrin deposits (white arrows). **c** Lymphadenitis of the prescapular lymph node (white arrows). **d** Congestion of the small intestine and blood vessels (white arrows)

the infection, while young animals suffered the most from acute respiratory distress, and five of six died a few days after infection, in agreement with the report of DaMassa et al. (1992). The observed mortality was due mainly to respiratory distress, but according to Laws et al., (1956), mycoplasma can present respiratory and digestive tropism at the same time. Parenteral or oral infection with Mcc is known to initiate definite progressive pathologic septicemia with severe joint localization, as reported by DaMassa et al. (1992). The mortality rate in our study is higher than that observed in the field and is probably due to the huge infection charge with a highly virulent strain. Mortality also occurred in adult sheep and goats, and the observed postmortem lesions were characterized by diffuse interstitial pneumonia, similar to the description of Rodríguez et al. 1998.

The farm contains only alpine goats, a breed highly sensitive to many infectious agents, as stated by several authors (Fakri et al. 2017; Bamouh et al. 2019). Although the disease has previously been reported in local breeds (Benkirane et al. 1993), experimental infection showed higher sensitivity of alpine goats (2/3 mortality at D6 and D8 pi) than local breeds (1/3 mortality at D14 pi). However, Mcc was considered less pathogenic for sheep than for goats (Perreau and Breard 1979). In this research, sheep were equally or more sensitive than goats, with a mortality rate of 5/6. Cases must be investigated in sheep, as also recommended by Taoudi et al., (1987).

Conclusions

This research describes for the first time the genetic characterization and pathogenicity of a North African

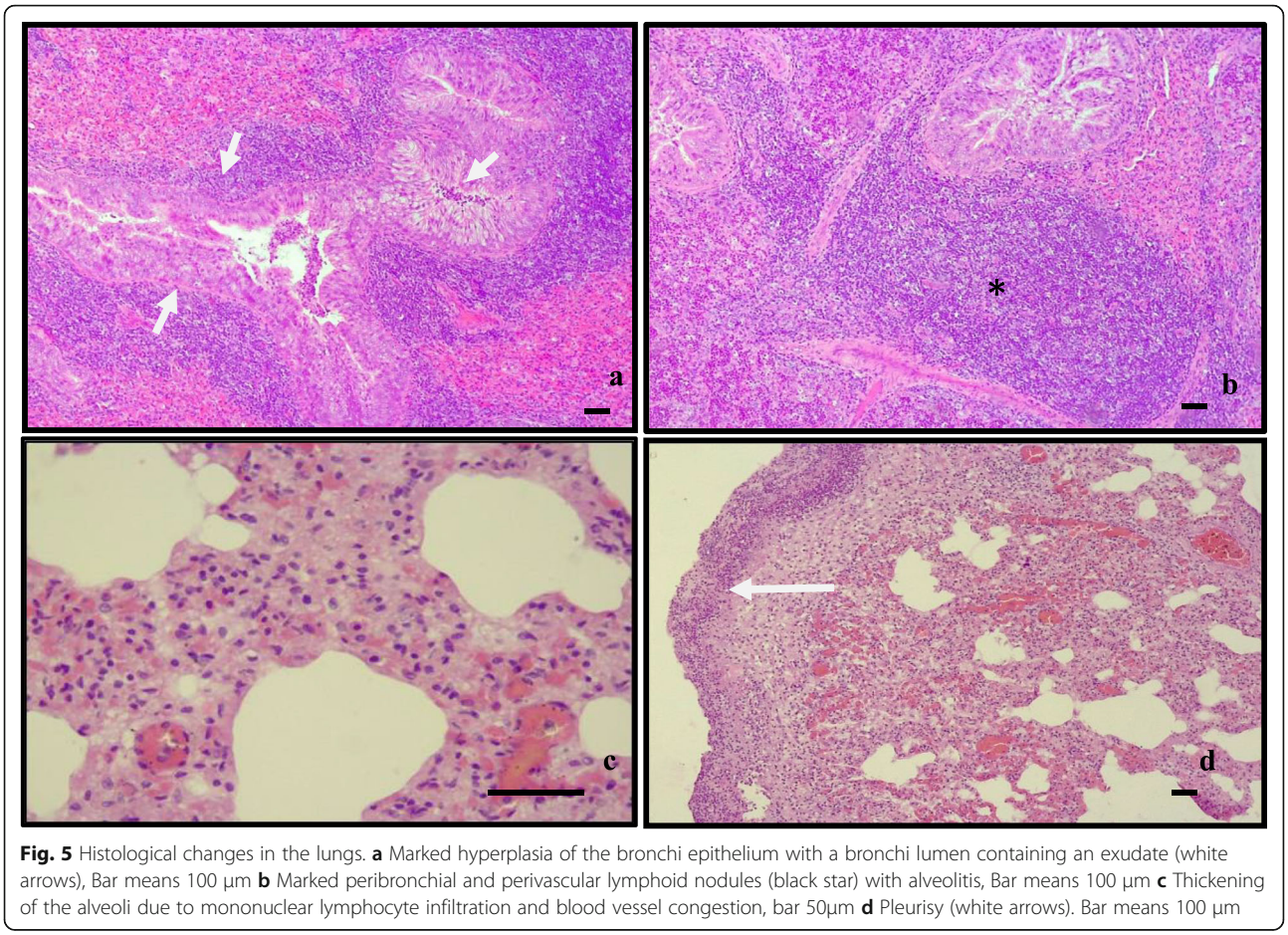

Fig. 5 Histological changes in the lungs. **a** Marked hyperplasia of the bronchi epithelium with a bronchi lumen containing an exudate (white arrows), Bar means 100 μm **b** Marked peribronchial and perivascular lymphoid nodules (black star) with alveolitis, Bar means 100 μm **c** Thickening of the alveoli due to mononuclear lymphocyte infiltration and blood vessel congestion, bar 50μm **d** Pleurisy (white arrows). Bar means 100 μm

isolate of Mcc. It provides evidence that contagious agalactia due to Mcc is a serious disease of goats and sheep, inducing economic losses by mortality of young animals and a reduction in productivity in adult subacute infection. The disease needs to be considered for prophylactic measure implementation by vaccination to control spread.

Methods

Study area

In the center of Morocco, the Marrakech region (32° 14′ N, 7° 57′ O), an intensive alpine goat flock of approximately 300 head of dairy production, was suspected of having contagious agalactia. Two lung samples were aseptically collected from animals dying; the section was realized in the interface between consolidated and unconsolidated areas and kept in sterile disposable plastic bags. Two milk samples and one synovial fluid were harvested aseptically from animals presenting mastitis and arthritis.

Bacterial isolation and identification

The surface of lung tissue with lesions was sterilized with a hot spatula, and deep tissue was minced with sterilized scissors. One gram of tissue was mixed with 9 mL of modified (PPLO) medium (BBL, Becton Dickinson, USA). Samples were cultivated for isolation of mycoplasma as described in the World Organization for Animal Health (OIE) Manual (2010). In brief, minced suspensions of lung tissues, milk (after vigorous shaking) and synovial fluid were tenfold serially diluted to 10^{-4} in PPLO broth and filtered through 0.45 μm. Each dilution was plated on PPLO agar and incubated at 37 °C in a humid atmosphere of 5% CO_2. The primary culture was purified by transferring a small isolated colony to PPLO broth and incubating at 37 °C for 3–5 d. Isolates were permissively identified based on colony morphology and PCR.

PCR identification

The DNA was extracted from 200 μL of purified bacterial culture using the isolate II genomic DNA Kit (Bioline, Italy) according to the manufacturer's protocol. Purified DNA of the Mcc reference strain (ATCC 27343) was used as a positive control. Mcc was identified by real-time PCR and confirmed by

LppA gene PCR. Real-time PCR amplification of Mcc was performed in a 20 μL reaction volume containing 10 μL, of Sensifast probe NO-Rox mix (2x), 1 μL of forward and reverse primers (10 μM) F: ATCATT TTTAATYCCTTCAAS 3', R: TACTATGAGTAATT ATAATATATGCAA 3, 0.5 μL of probe primer: Fam-ACAACAAGATGTATTAGCTTCAGG-Tamra, 0.4 μL of carboxyrhodamine (ROX) reference Dye, 4 μL of DNA template, and 3 μL of nuclease free water. The first step of the cycle parameter, named the denaturation step, was performed at 95 °C for 10 min, followed by 45 cycles of denaturation at 95 °C for 15 s and annealing/amplification at 56 °C for 1 min. The amplification was performed using Applied Biosystems™ 7500 Real-Time PCR. The LppA gene of *Mycoplasma capriculum subsp. capriculum* was amplified by PCR using the specific primers F: 5' AGAC CCAAATAAGCCATCCA 3' R: 5' CTTTCACCGCTT GTTGAATG 3' (Monnerat et al. 1999). PCR was carried out in a total volume of 25 μL containing 4 μL of genomic DNA (~ 15 to 25 ng), 1 μL of dNTPs (10 mM), 1 μL of each primer (10 mM), 1.25 μL of $MgCl_2$ (50 mM), 0.25 μL of Taq polymerase (5 U/μL) and 2.5 μL of PCR buffer (10x). Reactions were denatured for 5 min at 95 °C, and PCR amplification was subsequently carried out with 35 cycles at 95 °C for 30 s, 52 °C for 30 s, and 72 °C for 1 min each step. The results were visualized in 3% gel agarose with ethidium bromide. PCR amplifications of *Mycoplasma capricolum subsp. capripneumoniae*, *Mycoplasma mycoides subsp. capri*, *Mycoplasma putrefaciens* and *Mycoplasma agalactiae* were performed as described by Ying et al. (2011) and Kumar et al. (2011).

Sequencing and phylogenetic analysis

The sequencing of the LppA gene was carried out according to the Sanger method using a 16-capillary 3130XL genetic analyzer sequencer (Applied Biosystems). The gene sequence was analyzed and aligned with *LppA* gene sequence of Mcc ATCC 27343 by BioEdit. The FASTA sequence of *LppA* was compared with other sequences of the National Center for Biotechnology Information (NCBI) database by the Basic Local Alignment Search Tool. The conservation analysis of LppA amino acids was performed using the consurf web server, which estimates the evolutionary conservation of amino acids of proteins based on homology (Ashkenazy et al. 2016; Elkarhat et al. 2020). For construction of the phylogenetic tree, aligned individual nucleotide sequences of the deduced LppA gene were manually edited and concatenated together. The maximum likelihood tree was constructed from the artificially concatenated nucleotide sequences according to the amura-Nei model using the MEGA X program.

Serological analyses

From the affected goat herd, 80 blood samples were collected depending on the age of the animals. Sera were extracted and submitted for serology analysis by enzyme-linked immunoassay (ELISA). The test is based on microplates coated with Mcc lysate. The Mcc antigen was suspended in 100 μL of bicarbonate buffer bound to each well of a Maxisorp plate and incubated overnight at 4 °C. The plate was washed with phosphate-buffered saline (PBS). Then, goat and sheep collected sera diluted in 100 μL of blocking buffer were added to the plates. After 1 h of incubation at 37 °C, the plate was washed with PBS Tween and then incubated with 100 μL of horseradish peroxidase-conjugated anti-immunoglobulin G (Bethyl Laboratories) for an additional hour at 37 °C. Then, the plate was washed with PBS Tween, and the 3,3',5,5'-tetramethylbenidine dihydrochloride (TMB) substrate was added for 10-15 min at room temperature. The reaction was stopped by the addition of 50-100 μL of sulfuric acid per well, and the absorbance was read at 450 nm. Specific Mcc antibodies were assessed using the optical density (OD) as an indicator.

Experimental infection

Experiments were carried out according to international guidelines described for the care and handling of experimental animals, Chapter 7.8 of the Terrestrial Animal Health Code and Directive 2010/63/UE of the European Commission. The protocol was submitted and approved by the Internal Ethics Committee. Animals were maintained under observation during the experiment in animal boxes of Biosecurity level 3 containment.

Twelve goats and sheep were infected in this experiment and divided into four groups of three animals: G1 (local adult goats), G2 (alpine kid goats), G3 (local adult sheep) and G4 (local lambs). Before infection, animals tested negative for Mcc-specific antibodies by indirect ELISA. Animals were infected 4 times at 24 h intervals *via* the intratracheal route with the culture of Mcc isolate at a concentration of 10^8 colony-forming units (cfu) per mL as described by Taoudi et al. (1988). Clinical signs, feed intake, general behavior, rectal temperature, respiratory and cardiac frequency were monitored daily for two months after infection. For each individual, a score reflecting disease severity was calculated (Table 4).

Table 4 Scoring of recorded clinical signs

Clinical signs		Score
General behavior	Normal	**0**
	Inactive	**1**
	Low head	**2**
	Very inactive	**3**
	Very inactive + Hyperthermia	**4**
	Lying down	**5**
Respiratory symptoms	Normal	**0**
	Mucous nasal discharge	**1**
	Serous nasal discharge	**2**
	Purulent nasal discharge	**3**
	Nasal dischage + Cough	**4**
	Nasal dischage + Cough + Dispnea	**5**
Digestive symptoms	Normal	**0**
	Soft diarrhea	**1**
	Liquid diarrhea	**2**
	Profuse diarrhea	**3**
	Liquid diarrhea + Anorexia	**4**
	Profuse diarrhea + Anorexia	**5**
Lameness and keratoconjonctivitis	Normal	**0**
	Lameness of 1 member	**1**
	Lameness of 2 members	**2**
	Keratoconjontivitis	**3**
	Polyarthritis	**4**
	Lameness + Keratoconjonctivitis	**5**
Mortality		**5**

Histopathology

Necropsy was performed on dead or euthanized goats and sheep. Tissue specimens were collected and fixed in 10% neutral formalin and processed for histopathology. Paraffin tissue sections were cut at 5-μm thickness and stained with hematoxylin and eosin.

Abbreviations

BSL3: Biosafety level 3; ELISA: Enzyme-linked immunoassay; OIE: World Organization for Animal Health; RT-qPCR: Real-time quantitative polymerase chain reaction; Mcc: Mycoplasma capricolum subsp. capricolum; CA: Contagious agalactia; Mccp: Mycoplasma capricolum subsp. capripneumoniae; PCR: Polymerase chain reaction; PPLO: Pleuropneumonia-like organism.

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All the authors have seen and approved the content and have contributed significantly to the work. The authors gratefully acknowledge the support for this study by MCI (Multi-Chemical Industry) Santé animale and general manager Dr. Khalid Omari Tadlaoui.

Authors' contributions

All listed authors worked collectively on the design of the study and development of the review instruments. NS performed the experiment and data analysis and drafted the manuscript. ZZ collected field samples. ZE and SF carried out PCR testing and sequencing analysis. ZB and NS performed the isolation. NT carried out histopathology. OFF performed the study design, performed the followup of the study critically and reviewed the manuscript. ME supervised the study, data analysis, interpretation and manuscript drafting. All authors read and approved the final manuscript.

Funding

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Availability of data and materials

All data analyzed during this study are included in this published article. All recorded raw data are archived in MCI Santé Animale.

Declarations

Ethics approval and consent to participate

Animal experiments were carried out in accordance with the international guidelines for the care and handling of experimental animals. The study protocol was submitted and approved by the Internal Ethics Committee "The internal ethics committee for animal experiments, MCI santé animale". We obtained written consent from the MCI Santé Animale breeding farm to use the animals in the study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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