

Experimental infection of goats with an unusual strain of *Mycoplasma mycoides* subsp. *capri* isolated in Jordan: comparison of different diagnostic methods

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Summary

Ten goats were experimentally infected with a *Mycoplasma* identified by biomolecular methods as *Mycoplasma mycoides* subsp. *capri*, strain Irbid which was isolated from goats in an outbreak of contagious agalactia in north Jordan and defined as 'unusual', due to its serological characteristics. Two groups of goats infected by the endotracheal route and by aerosol, respectively, were placed in contact with a third group of naive animals. Six weeks after infection, some animals from both the infected and contact groups presented fever and nasal discharge, followed by severe respiratory signs and polyarthritis. Organs were taken from animals that died during the trial or those that were sacrificed at the end of the trial. The results of microbiological isolation and immunohistochemical tests conducted on the organs were compared after a description of the clinical picture and anatomopathological and histopathological signs.

Keywords

Diagnosis, Goat, Immunohistochemistry, Jordan, Microbiology, *Mycoplasma*, *Mycoplasma mycoides* subsp. *capri*.

Introduction

The mycoplasmas of the *mycoides* cluster are pathogenic agents of cattle, goats and sheep. *Mycoplasma mycoides* subsp. *mycoides*, large colony type (*Mmm*LC) and *Mycoplasma capricolum* subsp. *capricolum* cause septicaemia, arthritis, mastitis and pneumonia in small ruminants, while *Mycoplasma mycoides* subsp. *capri* (*Mmc*) is the primary cause of pneumonia in goats (16).

*Mmm*LC and *Mmc* are genetically indistinguishable, causing the International Committee on Systematics of Prokaryotes, Subcommittee on the Taxonomy of the *Mollicutes*, to evaluate a proposal to group *Mmm*LC and *Mmc* as a single subspecies, called *Mycoplasma mycoides* subsp. *capri*. For this reason, the name *Mycoplasma mycoides* subsp. *capri* will be used hereafter.

Mmc is one of the most widespread pathogens in countries in which small ruminants are farmed (6, 11); in outbreaks of disease, mortality can reach 90% (15). Its presence and impact on livestock are probably underestimated, as not all countries are able to diagnose *Mycoplasma* infections (19).

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Little is known about the presence of mycoplasmosis in the Middle East. In north Jordan, mycoplasmas from the *Mycoides* cluster have been isolated in sheep and goats suffering from pneumonia and signs attributable to contagious agalactia; these included the Irbid strain, which, despite having the same genetic features as *Mmc*, shows no growth inhibition on contact with reference anti-*Mmc*- and anti-*Mmm*LC antisera (1, 7).

In goats infected experimentally via the respiratory route, fever, anorexia, mild conjunctivitis, joint swelling and pulmonary lesions were observed (7).

The aim of this study was to determine the distribution of *Mmc* in the various tissues of animals experimentally and naturally infected and to compare the diagnostic sensitivity of microbiological isolation (MI) and immunohistochemistry (IHC) testing.

Materials and methods

Infection of goats

The *Mmc* Irbid strain used for the infection was identified by polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) (4, 9). A pure culture of *Mycoplasma* at a concentration of 10^8 colony-forming units (cfu) per ml was used for the infection (7).

Ten healthy female goats of a Saanen × Chamois × Garganica crossbreed were used; they were all aged between 1 and 2 years and came from the same farm. The goats had tested seronegative for *Mmc* by the complement fixation test, enzyme-linked immunosorbent assay (ELISA) and latex agglutination test (LAT) (3). Nasal swabs tested for *Mycoplasma* spp. by bacteriological assay and PCR (8, 12) proved to be negative.

Animals were housed in the high-security stables at the *Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale'* (IZS A&M) in Teramo. The trial was conducted in compliance with Italian legislation on animal welfare (2).

The goats were randomly divided into three groups in agreement with the experimental design as follows:

- **Group 1**, consisting of two animals infected using an endotracheal tube with 10 ml of *Mmc* suspension (IET) (10, 18)
- **Group 2**, consisting of three animals infected via the respiratory route by non-sonicated aerosol (Mister Baby 'Family' range) with the same quantity of inoculum as used for the first group (AER); the infectious agent was nebulised in a room that was physically separated from the stable in which animals were housed together after the infection.
- **Group 3**, consisting of five animals placed in contact with the other two groups on the day on which the latter were infected, with the aim of inducing natural disease transmission (CONT).

Animals in groups 1 and 2 were infected using an anaesthetic protocol involving a mixture of 0.2 mg/kg of xylazine hydrochloride (Bayer) and 1 mg/kg of ketamine hydrochloride (Intervet) inoculated via intravenous catheter into the right jugular vein. A solution of local anaesthetic (lidocaine hydrochloride 2%) (Fort Dodge) was also used in group 1, to reduce the swallowing reflex. Animals were sacrificed under general anaesthetic by intravenous inoculation of Tanax®, under veterinary supervision.

Sampling

Animals were sacrificed on presentation of clinical signs, or in their absence, were slaughtered 56 days post-infection or post-contact in accordance with the experimental protocol which included the slaughter of animals at two months post infection.

Samples were taken of the apical, cardiac, diaphragmatic and accessory lobes from the right and left lungs, from the heart, liver, kidney, spleen, carpal and metacarpal joint tissue, uterus, vagina, placenta and breast. In addition, samples were taken from pre-scapular, retropharyngeal, iliac, mediastinal, peribronchial and supramammary lymph nodes.

Microbiological isolation

The isolation was conducted on tissue samples of about 1.5 cm³, taken under sterile conditions (12). The samples were processed using the following procedure:

- material was suspended in 10 ml of tryptose broth, then mechanically homogenised with a Stomacher®
- the homogenate was placed in sterile V-bottomed test tubes and centrifuged at 1 400 × g for 15 min at 4°C.

After centrifugation, the supernatant was collected with a syringe and filtered using Millipore 45 µm filters, 10 drops of filtrates were seeded in broth and on contagious caprine pleuropneumonia (CCPP) agar medium (20). Cultures were incubated at 37°C under 5-10% CO₂ for 7 days. Both agar plates and broths were checked every day for any mycoplasma growth. Where plates were negative and growth was found in the culture broths, 10 drops of the broth were inoculated onto the CCPP agar plates and incubated as above.

Positive samples underwent molecular typing by PCR (8).

Histology

Organ tissue samples were fixed in 10% neutral buffered formalin. The fixed samples were dehydrated under vacuum and imbedded in paraffin, to obtain 5 µm-thick sections stained with haematoxylin and eosin (H&E).

Immunohistochemistry

Immunohistochemical staining was performed using the 'streptavidin biotin complex peroxidase' (StreptABC-Perox) kit (Dako) on all histological lung and lymph node sections and generally on histological sections of organs that tested positive for isolation of the aetiological agent. A hyperimmune rabbit serum produced from the same strain used for the infection (IZS A&M) and diluted 1:200 in 0.15 M Tris solution buffered to pH 7.6 containing 5% skimmed milk powder was used as the primary antibody (13). *Mmc*-negative rabbit serum (IZS A&M) was used for the negative controls. The biotin-conjugated

monoclonal antibody clone RG-96 (Sigma) was used as a secondary antibody.

Results

Clinical and anatomopathological signs

All animals in group 1 presented severe respiratory signs and clear anatomopathological lung and joint lesions. Goat 1 was sacrificed 30 days after infection on presentation of respiratory signs, while goat 2 died on day 24, presenting lymphadenitis as well as respiratory signs.

In group 2 only goat 4, which died on day 44, showed respiratory signs, while goats 3 and 5, both sacrificed on day 56, presented lameness and slight nasal discharge, respectively. Only goat 5 presented lesions attributable to pneumonia, pleurisy and lymphadenitis.

Goats from group 3 were sacrificed on day 56, with the exception of goats 9 and 10, which had died on days 38 and 43, respectively. Goats 8, 9 and 10 presented respiratory signs with coughing, while goats 6 and 7 presented nasal discharge only. On autopsy, sero-fibrinous pneumonia, differing in severity and involving different lung lobes, was found in 8 out of 10 animals, while goat 9 also presented pleurisy, pericarditis and lymphadenitis; the latter was also found in goat 10.

The clinical and anatomopathological findings are summarised in Table I.

Histology

When present, histological lesions were mainly found in the lungs.

In group 1, goat 1 presented bronchial and alveolar fibrinous-catarrhal inflammation, septal fibrosis (Fig. 1), necrotic foci (Fig. 2) and coagulative necrosis, mainly on the right apical and cardiac lobes; goat 2 presented chronic fibrinous pleurisy (Fig. 3), necrotising pneumonia, newly formed capillaries and fibrinous pericarditis, with acute granulocytic inflammation.

In group 2, goat 4 presented interstitial alveolitis and serofibrinous exudation, with inflammatory cells in the alveoli and

Table I
Clinical signs and anatomopathological lesions

Group and goat number	Infection method	Days of fever >40°C	Clinical signs	Days from infection to death/sacrifice	Anatomopathological lesions					
					Pleural fluid	Peri-cardial fluid	Peri-carditis	Pneumonia	Poly-arthritis	Lymphadenitis
1 / 1	IET	14-21	Respiratory, coughing, lameness	30	+	+	+	+	+	-
1 / 2	IET	15-21	Respiratory, coughing, lameness	24	+	+	+	+	+	+
2 / 3	AER	No	Lameness	56	-	-	-	-	-	-
2 / 4	AER	No	Respiratory	44	-	+	-	-	-	+
2 / 5	AER	16-21	Slight nasal discharge	56	+	+	-	+	-	+
3 / 6	CON	No	Slight nasal discharge	56	-	-	-	+	-	+
3 / 7	CON	14-17	Slight nasal discharge	56	-	-	-	+	-	-
3 / 8	CON	No	Mild respiratory, coughing	56	-	-	-	+	-	-
3 / 9	CON	32-38	Respiratory, coughing	38	+	-	+	+	-	+
3 / 10	CON	37-43	Respiratory, coughing	43	-	-	-	+	-	+

IET infected using an endotracheal tube
AER respiratory route by non-sonicated aerosol
CON infection by contact

+ presence
- absence

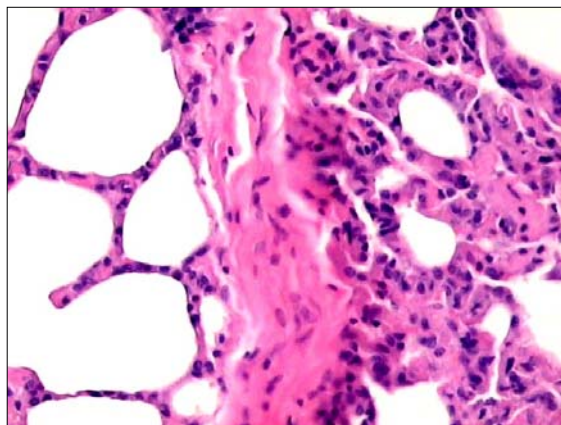


Figure 1
Lung: septal fibrosis in the centre, an area of alveolar atelectasis on the right and an area of alveolar vicarious emphysema on the left
Goat no. 1 (group 1)
(haematoxylin and eosin, 40×)

bronchioles (Fig. 4) and small areas of hyperplasia of the bronchus-associated lymphoid tissue (BALT) (Fig. 5); goats 3 and 5 did not show significant lesions, despite the

fact that macroscopic pulmonary lesions were observed in animal 5.

In group 3, goat 9 presented septal fibrosis, desquamative macrophagic alveolitis, interlobular oedema and compensatory emphysema,

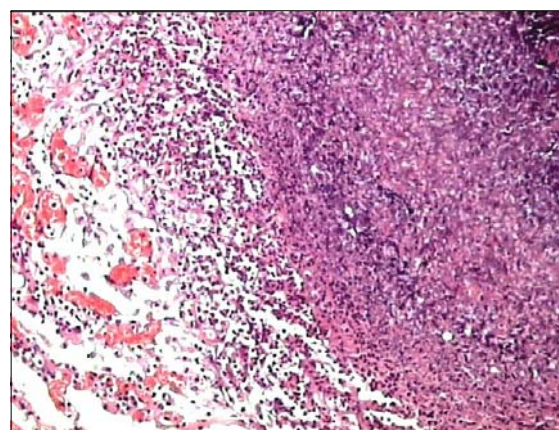


Figure 2
Lung: necrotic area with degenerated inflammatory cells
Goat no. 1 (group 1)
(haematoxylin and eosin, 10×)

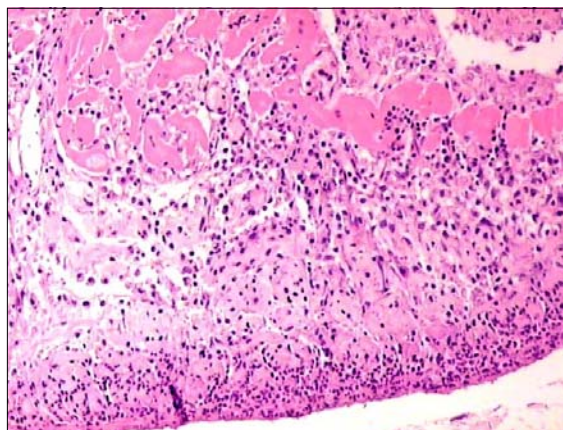


Figure 3
Lung: fibrinous pleurisy
Goat no. 2 (group 1)
(haematoxylin and eosin, 10×)

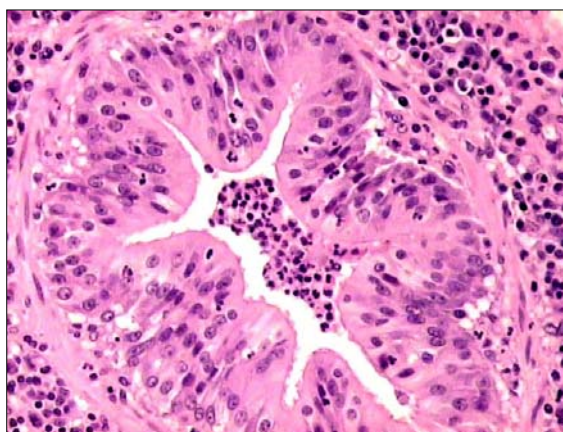


Figure 4
Lung: sero-fibrinous exudate and inflammatory
cells in bronchioles
Goat no. 4 (group 2)
(haematoxylin and eosin, 10×)

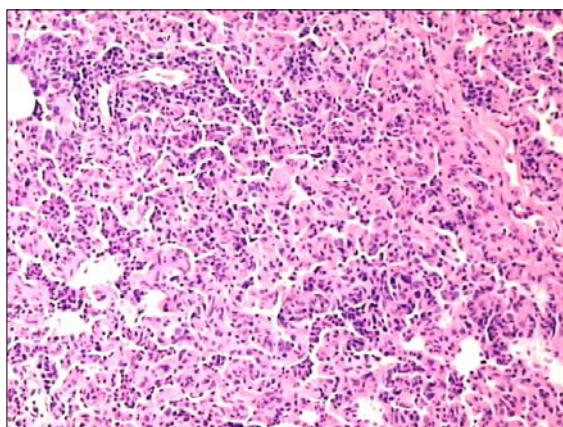


Figure 5
Lung: hyperplasia of the bronchus-associated
lymphoid tissue
Goat no. 4 (group 2)
(haematoxylin and eosin, 10×)

atelectasis, serofibrinous bronchiolitis, fibrinous pleurisy and mediastinal lymph node hyperplasia. No significant lesions were found in goats 6, 7, 8 and 10.

No lesions attributable to mycoplasma infection were found in the other organs examined.

Isolation and immunohistochemistry

With the exception of goat 5, *Mmc* was isolated (Fig. 6) from all animals of three groups, in which pulmonary lesions were found, as noted above.

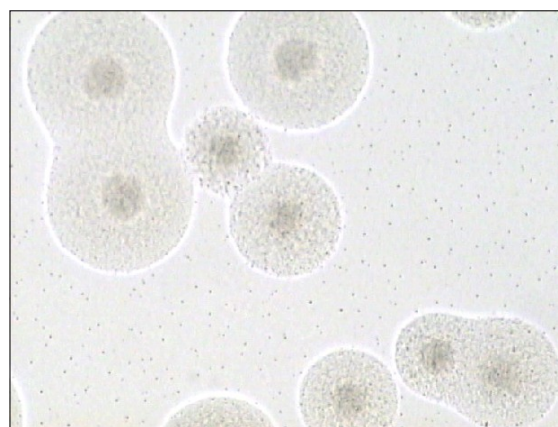


Figure 6
Mycoplasma mycoides subsp. *capri* used for
experimental infection in contagious caprine
pleuropneumonia agar
(10×)

In group 1, immunohistochemical, intracellular, and extracellular localisation revealed *Mmc* in the lungs (necrotic areas) (Fig. 7), alveolar and bronchiolar lumens (Fig. 8), alveolar and bronchiolar epithelium (Fig. 9), pleura (Fig. 10) and heart (inflammatory granulocytic infiltrate) of goat 2 only (Fig. 11).

In group 2, *Mmc* was isolated only from the retropharyngeal lymph nodes of goats 3 and 4, while antigen testing was only positive in the pulmonary catarrhal exudate, bronchiolar and alveolar lumens and bronchiolar epithelium of goat 4. The organs of goat 5 were negative on both tests.

In group 3, immunohistochemical localisation of *Mmc* was found only in the lungs of goats 9 and 10, in the necrotic areas, in the macrophage and granulocyte neutrophil

cytoplasm (Figs 12 and 13) in the subpleural inflammatory infiltrate and in the BALT.

The results of the isolation and immunohistochemical localisation of *Mycoplasma* are compared in Table II.

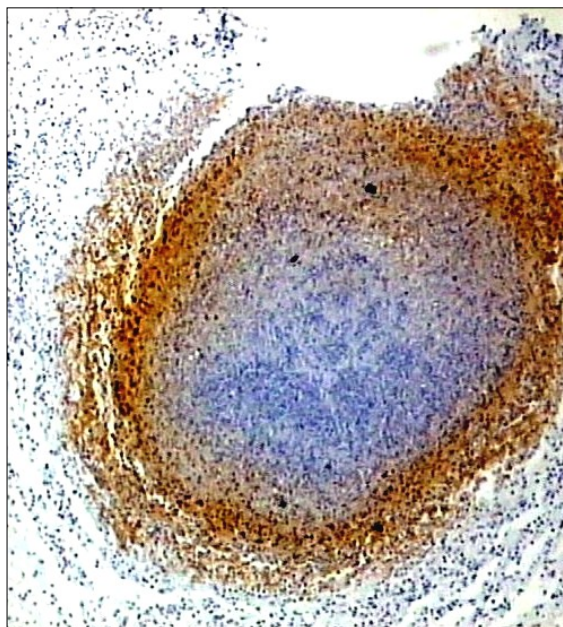


Figure 7
Lung: positive immunohistochemical staining in the area of necrosis
Goat no. 2 (group 1)
(streptavidin biotin complex peroxidase, 5×)

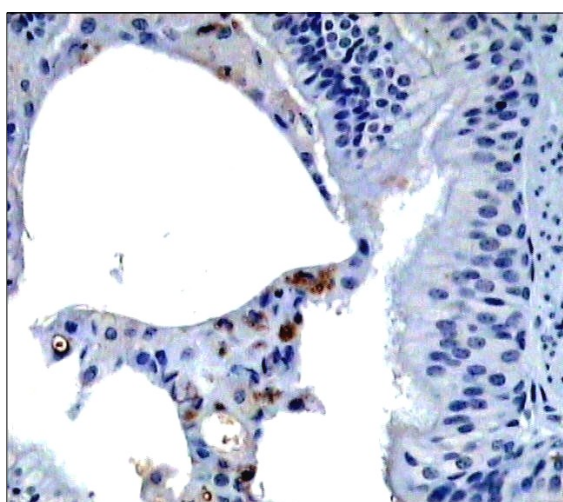


Figure 8
Lung: positive immunohistochemical staining of cells in the catarrhal exudate in the bronchiolar lumen
Goat no. 2 (group 1)
(streptavidin biotin complex peroxidase, 20×)

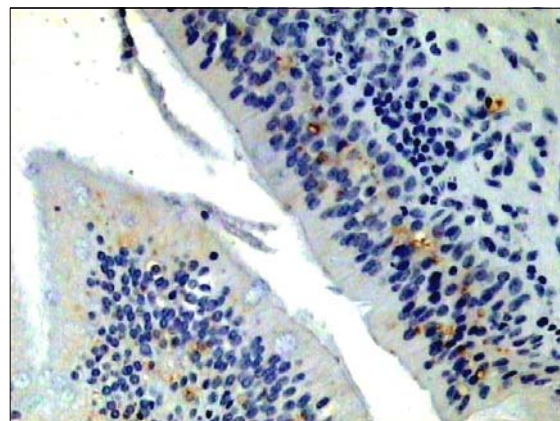


Figure 9
Lung: positive immunohistochemical staining in the bronchiolar epithelial cells
Goat no. 2 (group 1)
(streptavidin biotin complex peroxidase, 20×)

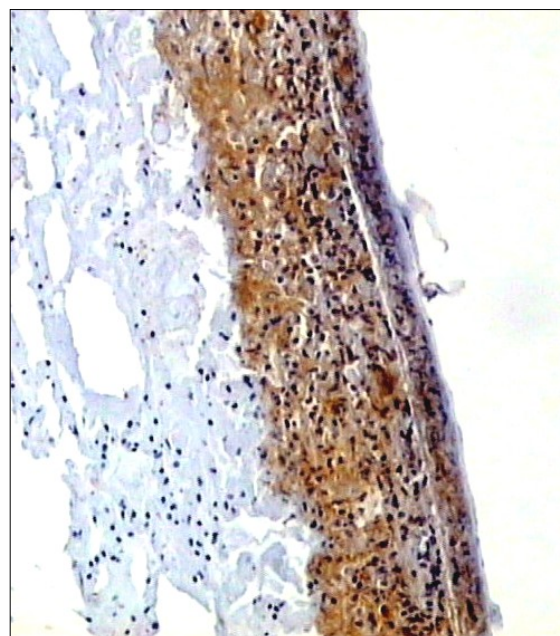


Figure 10
Lung: positive immunohistochemical staining in the sub pleural inflammatory infiltration
Goat no. 2 (group 1)
(streptavidin biotin complex peroxidase, 10×)

Discussion

Histological investigations revealed lesions that, although typical of respiratory mycoplasmosis in goats, cannot be defined as pathognomonic (5, 14, 17).

Both MI and IHC localisation of *Mycoplasma* revealed the lung to be the target organ; sampling of various lung areas naturally increased the chance of diagnostic success. The

retropharyngeal, mediastinal and peri-bronchial lymph nodes were also found to be target areas for microbiological isolation.

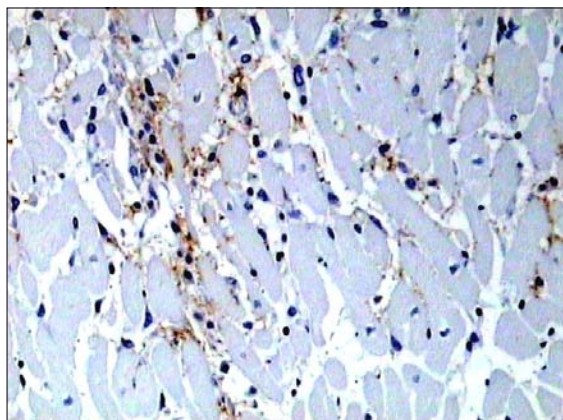


Figure 11
Heart: positive immunohistochemical staining in the granulocytic inflammatory infiltration
Goat no. 2 (group 1)
(streptavidin biotin complex peroxidase, 20×)

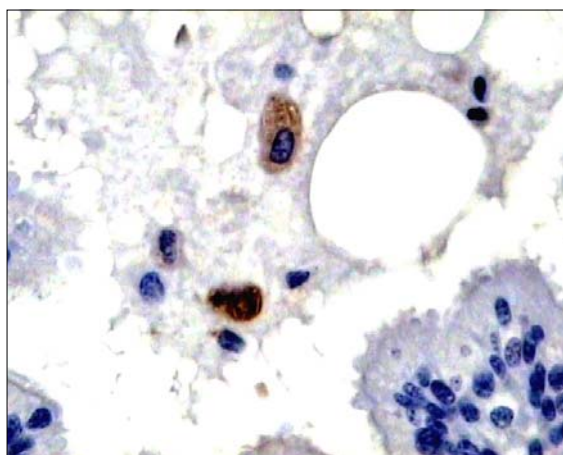


Figure 12
Lung: positive immunohistochemical staining in the cytoplasm of macrophages
Goat no. 9 (group 3)
(streptavidin biotin complex peroxidase, 40×)

In organs found positive on both MI and IHC testing, the antigen was localised in the cytoplasm of the inflammatory cells, especially the macrophages, and in the necrotic areas, as described above (14, 16, 17). Infiltration of the cytoplasm results in activation by the *Mycoplasma* of the phagocytic cells, followed in the more advanced phases by the release of fragments of *Mycoplasma* in the necrotic areas, originating from macrophage enzyme

digestion. Indeed, IHC staining can reveal not only whole micro-organisms, but also fragments, as long as they preserve their antigenicity. This would explain why the lung of goat 4 was found negative on MI but positive on IHC testing.

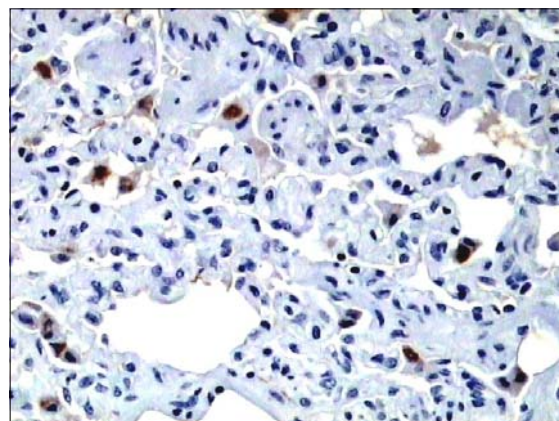


Figure 13
Lung: positive immunohistochemical staining of the granulocytes
Goat no. 10 (group 3)
(streptavidin biotin complex peroxidase, 20×)

A positive result on microbiological isolation, combined with a lack of immunohistochemical detection of *Mmc*, is indicative of the lower sensitivity of the latter test.

Conclusions

It can be concluded that the two test techniques when used together boosted diagnostic sensitivity, providing an overall evaluation of a farm's infection status. As it does not exploit any antigen properties, MI has the benefit of enabling diagnosis even in the presence of unusual strains of *Mycoplasma*, as in this case.

When the micro-organism is present, even in a non-viable form, IHC testing is effective and enables the study of the localisation of the pathogen in various cell populations, making it a useful tool in understanding the pathogenesis of the disease.

Table II
Results of microbiological isolation and immunohistochemistry
The lilac boxes show the positive reactions to microbiological isolation
The violet boxes show the positive reactions to immunohistochemical staining

Organs tested	Group 1		Group 2						Group 3											
	Goat no. and day of death or sacrifice post infection		Goat no. and day of death or sacrifice post infection		Goat no. and day of death or sacrifice post infection		Goat no. and day of death or sacrifice post infection		Goat no. and day of death or sacrifice post infection		Goat no. and day of death or sacrifice post infection		Goat no. and day of death or sacrifice post infection							
	1 30 days	2 24 days	3 63 days	4 44 days	5 63 days	6 63 days	7 63 days	8 63 days	9 38 days	10 43 days	MI	IHC	MI	IHC						
Right apical lobe	+	-	+	+	-	-	-	+	-	-	-	-	+	-	-	-	+	-	+	+
Left apical lobe	-	-	+	-	-	-	-	+	-	-	+	-	-	-	-	-	+	+	+	-
Right diaphragmatic lobe	-	-	+	+	-	-	-	+	-	-	+	-	+	-	-	-	+	-	+	+
Left diaphragmatic lobe	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-
Right cardiac lobe	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	+	+	+	-
Left cardiac lobe	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	+	-	+	-
Right accessory lobe	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	+	-	+	-
Right mid lobe	+	-	-	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
Mediastinal lymph node	-	-	+	+	/	/	/	/	/	/	-	-	-	-	-	-	+	-	+	/
Peribronchial lymph node	-	-	+	-	-	-	-	/	-	/	-	/	-	/	-	/	+	-	+	/
Prescapular lymph node	-	-	+	-	/	/	/	/	/	/	-	/	-	/	-	/	+	-	+	-
Retropharyngeal lymph node	/	/	+	/	+	-	+	-	-	/	-	-	-	/	+	/	+	-	-	/
Iliac lymph node	/	/	/	/	/	/	/	/	/	/	-	/	-	/	-	/	+	-	-	/
Heart	-	-	+	+	/	/	/	/	/	/	-	/	-	/	-	/	-	/	/	/
Pericardium	-	-	+	+	/	/	/	/	/	/	-	/	-	/	-	/	+	-	-	/
Liver	-	-	-	/	/	/	/	/	/	/	-	/	-	/	-	/	-	/	+	-
Spleen	-	-	-	/	/	/	/	/	/	/	-	/	-	/	-	/	-	-	+	-
Kidneys	-	-	-	/	/	/	/	/	/	/	-	/	-	/	-	/	+	-	+	-
Breast	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	+	/	/	/
Vagina	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	+	/	/	/
Uterus	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	+	/	/	/
Placenta	-	-	-	/	/	/	/	/	/	/	-	/	-	/	-	/	+	-	-	/
Joints	+	-	+	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/

MI microbiological isolation
IHC immunohistochemistry testing
+ presence
- absence
/ not conducted

Finally, use of the IHC technique should be considered in climatic and environmental conditions that are unfavourable for microbiological isolation, such as those found in some areas of Africa and Asia. The poor resistance of *Mycoplasma* in the external environment, the extreme difficulty in

maintaining refrigeration temperatures during transportation and the often considerable distances between the site of sampling and the laboratory are elements that point towards the use of the IHC technique, which requires nothing but fixing in 10% formalin to conserve the sample.

References

1. Al-Momani W. & Nicholas R.A.J. 2006. Small ruminant mycoplasmas with particular reference to the Middle East. *Vet Bull*, **76**, 1-11.
2. Anon. 1992. Decreto legislativo 27 gennaio 1992, n.116. Attuazione della direttiva (CEE) n. 609/86 in materia di protezione degli animali utilizzati a fini sperimentali o ad altri fini scientifici. Suppl. ordinario alla *Gazz Uff*, **40**, 18 febbraio (www.unife.it/ricerca/ricerca-nazionale/tutela-animali-utilizzati-a-fini-sperimentali/decreto-legislativo-27-gennaio-1992-n.116 accessed on 7 June 2010).
3. Ayling R.D., Regalla J. & Nicholas R.A.J. 1999. A field test for detecting antibodies to *Mycoplasma mycoides* subsp. *mycoides* SC using the latex slide agglutination test. In *Mycoplasmas of ruminants: pathogenicity, diagnostics, epidemiology and molecular genetics*, Vol. 3 (L. Stipkovitz, R. Rosengarten & J. Frey, eds). EC. EC Brussels, EUR 18756, 155-158.
4. Bashiruddin J.B. 1998. PCR and RFLP methods for the specific detection and identification of *Mycoplasma mycoides* subsp. *mycoides* SC. In *Mycoplasma protocols* (R.J. Miles & R.A.J. Nicholas, eds). Humana Press, Totowa, 37-44.
5. Cottew G.S. 1979. Pathogenicity of the subspecies *mycoides* of *Mycoplasma mycoides* for cattle, sheep and goats. *Zentralbl Bakteriol Orig A*, **245** (1-2), 164-170.
6. DaMassa A.J., Wakenell P.S. & Brooks D.L. 1992. Mycoplasmas of goats and sheep. *J Vet Diag Invest*, **4** (1), 101-113.
7. Di Provvio A., Scacchia M., Varasano V., Churchward C., De Caro C., Al-Momani W., Di Francesco G., Ayling R.D., Lelli R. & Nicholas R.A. 2009. Experimental infection of goats with an unusual strain of *Mycoplasma mycoides* subsp. *capri* isolated in Jordan. *J Comp Pathol*, **14**, 121-126.
8. Hotzel H., Sachse K. & Pftzner H. 1996. A PCR scheme for differentiation of organisms belonging to the *Mycoplasma mycoides* cluster. *Vet Microbiol*, **49** (1-2), 31-43.
9. McAuliffe L., Ellis R., Lawes J., Ayling R.D. & Nicholas R.A.J. 2005. 16S r DNA and DGGE: a single generic test for detecting and differentiating *Mycoplasma* species. *J Med Microbiol*, **54**, 1-9.
10. March J.B., Harrison J.C. & Borich S.M. 2002. Humoral immune responses following experimental infection of goats with *Mycoplasma capricolum* subsp. *capripneumoniae*. *Vet Microbiol*, **84** (1-2), 29-45.
11. Nicholas R.A.J. 2002. Improvements in the diagnosis and control of diseases of small ruminants caused by mycoplasmas. *Small Ruminant Res*, **45**, 145-149.
12. Nicholas R.A.J. & Baker S.E. 1998. Recovery of mycoplasmas from animals. In *Mycoplasma protocols* (R.J. Miles & R.A.J. Nicholas, eds). Humana Press, Totowa, 37-44.
13. Prophet E.B., Mills B., Arrington J.B. & Sobin L.H. 1994. Immunohistochemistry. American Registry of Pathology, Washington, DC, Volume 23, 247-255.
14. Rodriguez F., Kennedy S., Bryson T.D., Fernandez A., Rodriguez J.L. & Ball H.J. 1996. An immunohistochemical method of detecting *Mycoplasma* species antigens by use of monoclonal antibodies on paraffin sections of pneumonic bovine and caprine lungs. *Zentralbl Veterinarmed B*, **43** (7), 429-438.
15. Rodriguez J.L., Poveda J.B., Oros J., Herraes P., Sierra M.A. & Fernandez A. 1995. High mortality in goats associated with the isolation of a strain of *Mycoplasma mycoides* subsp. *mycoides* (large colony type). *Zentralbl Veterinarmed B*, **42** (10), 587-593.
16. Rodriguez J.L., DaMassa A.J., Oros J., Rodriguez F., Herraes P. & Fernandez A. 1997. Immunohistochemical and microbiological study of the oropharyngeal region of goats experimentally infected with *Mycoplasma mycoides* cluster strains. *Aust Vet J*, **75** (10), 753-755.
17. Rodriguez J.L., Gutierrez C., Brooks D.L., DaMassa A.J., Oros J. & Fernandez A. 1998. A pathological and immunohistochemical study of goat kids undergoing septicaemic disease caused by *Mycoplasma capricolum* subsp. *capricolum*, *Mycoplasma mycoides* subsp. *capri* and *Mycoplasma mycoides* subsp. *mycoides* (large colony type). *Zentralbl Veterinarmed B*, **45** (3), 141-149.
18. Rosendal S. 1983. Susceptibility of goats and calves after experimental inoculation or contact exposure to a Canadian strain of *Mycoplasma mycoides* subsp. *mycoides* isolated from a goat. *Can J Comp Med*, **47** (4), 484-490.
19. World Organisation for Animal Health (Office International des Épidémiologies: OIE) 2008. Contagious agalactia. In *Manual of diagnostic tests and vaccines for terrestrial animals*. OIE, Paris, 992-999.
20. World Organisation for Animal Health (Office International des Épidémiologies: OIE) 2008. Contagious caprine pleuropneumonia. In *Manual of diagnostic tests and vaccines for terrestrial animals*. OIE, Paris, 1000-1002.