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Mycoplasma bovis infection in respiratory disease of dairy calves less than one month old

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ABSTRACT

Mycoplasma bovis is an important cause of bovine respiratory disease, especially in young calves where it can also cause arthritis, tenosynovitis and otitis. During 2009 and 2010 a survey was carried out on carcasses of calves less than one month old sent to the Diagnostic Laboratory of the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna in Brescia, regardless of the presence of lung lesions, to detect this pathogen. PCR tests for *Mycoplasma* spp. and *M. bovis* were applied. 83 out of 224 (37%) lung tissue samples examined were positive at PCR test for *Mycoplasma* spp.; in 64 cases of these we observed typical respiratory lesions ($P < 0.001$). *M. bovis* was identified in 26 out of 83 (31%) lung tissue samples positive at PCR test for *Mycoplasma* spp.; in 24 cases of these we observed typical respiratory lesions ($P = 0.039$). Our data demonstrate that presence of *Mycoplasma* spp. and *M. bovis* positively correlates with pneumonic lung lesions in young dairy calves.

1. Introduction

Mycoplasmas, which are the smallest prokaryotes capable of independent life, are classified in *Mollicutes* Class, and many of the species that affect animal pathologies are included in *Mycoplasmatales* Order, *Mycoplasmataceae* Family and *Mycoplasma* Genus (Farina et al., 1998).

To date, about 40 *Mycoplasma* species have been described in domestic ruminants (bovine, ovine, goats), some of which are pathogenic. Three *Mycoplasma*-induced ruminant diseases are currently listed by the World Organization for Animal Health (OIE) to be of major economic importance: contagious bovine pleuropneumonia caused by *Mycoplasma mycoides* subsp. *mycoides* SC (SC = small colonies), contagious caprine pleuropneumonia caused by *Mycoplasma capricolum* subsp. *capripneumoniae*, and contagious agalactia affecting sheep and goats, caused by several *Mycoplasma* species, but mainly by *Mycoplasma agalactiae*, *M. mycoides* subsp. *capri*, *M. capricolum* subsp. *capricolum* and *Mycoplasma putrefacien* (Chazel et al., 2010).

Mycoplasma bovis is recognized worldwide as an important cause of respiratory disease, arthritis and tenosynovitis in feedlot cattle and young dairy and veal calves. As well as acute respiratory disease it also causes chronic bronchopneumonia with caseous and coagulative necrosis, characterized by persistent infection that responds poorly to many antibiotics (Caswell and Archambault, 2007).

M. bovis can be a primary pathogen, but it often acts in co-infection with other microorganisms (bacteria and/or viruses), colonizing lung lesions initiated by other infective agents such as *Mannheimia haemolytica* (Caswell et al., 2010).

M. bovis prevalence has been studied by many authors from various countries, but limited data are available concerning the impact of *M. bovis* in calves less than one month old.

Northern Italian Regions, particularly Piedmont, Lombardy, Veneto and Emilia Romagna, are characterized by a high concentration of dairy farms; in these Regions cattle is constantly monitored for bovine infections (brucellosis, tuberculosis, enzootic bovine leukosis, paratuberculosis, IBR) by National or Regional Control Programs, whose aims are the control, eradication and surveillance of these diseases.

However, in these same regions little is known about the prevalence and epidemiology of many other serious bovine diseases, including the *M. bovis* infection, which can cause severe economic loss. The aim of this study was to investigate the presence of *M. bovis* in young dairy calves (under one month of age) and its correlation with pathological lesions observed at necropsy.

2. Materials and methods

All calf carcasses sent to the Diagnostic Laboratory of the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna in Brescia during standard diagnostic routine are normally submitted to a series of well-defined tests, according to diagnostic protocols previously developed by the Veterinarians working in this Laboratory.

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During 2009 and 2010, regardless of lesions observed and protocols applied, specific lung tissue specimens were taken from calves under one month of age carcasses.

All lung specimens were submitted to PCR test for *Mycoplasma* spp. detection and, if positive, to another PCR test specific for *M. bovis* (Foddai et al., 2005); specimens that were positive to *Mycoplasma* spp. were also submitted to bacteriological tests using specific media for *Mycoplasma* culture.

These specimens were also submitted to a series of specific tests for the identification of different respiratory pathogens. In particular, bacteriological and virological cultures from lung tissue and immunofluorescence tests for respiratory viral diseases from lung and tracheal tissue were carried out. Some lung specimens were also submitted to PCR test for *Histophilus somni* identification.

The PCR tests for *Mycoplasma* spp. and *M. bovis* and the bacteriological culture techniques for *Mycoplasma* spp. used in this study are illustrated below.

3. PCR test

3.1. Specimen preparation

Lung tissue (2–3 g) was homogenized and diluted 1/10 in MEM medium.

3.2. DNA extraction

150 μl of prepared material were added to an Eppendorf micro-tube containing 500 μl guanidine thiocyanate 4 M pH 7.5 + 1% mercaptoethanol, 500 μl phenol ph 6.7 and 200 μl chloroform:isoamilic alcohol = 24:1. The micro-tube was then shaken on a vortex for 20 s and centrifuged at 13,000 rpm at 4 °C for 15 min; 500 μl of supernatant containing nucleic acids was transferred to another Eppendorf micro-tube containing 500 μl isopropanol and 50 μl sodium acetate 3 M pH 5.2 and incubated at −20 °C overnight. The next day the specimen was centrifuged at 13,000 rpm at 4 °C for 30 min; the pellet was washed with 300 μl ethanol 70%, centrifuged at 13,000 rpm at 4 °C for 10 min. and re-suspended in distilled water + DEPC (diethylpyrocarbonate).

3.3. Amplification for Mycoplasma spp.

Primers:

Primer A: 5′-ACT CCT ACG GGA GGC AGC AGT A-3′
Primer B: 5′-TGC ACC ATC TGT CAC TCT GTT AAC CT-3′
Amplified fragment: 771 bp

3.4. Amplification for M. bovis

Primers:

mb-mp 1F: 5′-TAT TGG ATC AAC TGC TGG AT-3′
mb-mp 1R: 5′-AGA TGC TCC ACT TAT CTT AG-3′
Amplified fragment: 447 bp

3.5. Electrophoresis

3.5.1. Loading of PCR specimens

17 μl of specimen were added with 2 μl of blue dye and loaded on agarose gel 2% together with molecular weight marker; electrophoretic run takes place at 100 V for 20–30 min, evidenced by UV lamp and photographed.

4. Microbiological tests

All lung specimens positive for *Mycoplasma* spp. PCR test were submitted to microbiological test using the following method.

4.1. Bacteriological tests

PPLO agar and PPLO broth were used as culture media. Lung specimens plated on agar (undiluted) were incubated at 37 °C for 5–6 days at 10% CO_2 atmosphere. Specimens inoculated in broth were previously diluted 1/10 until $1/10^{-5}$ dilution and each dilution was inoculated in broth and incubated at 37 °C for 5–6 days at 10% CO_2 atmosphere. Agar plates were read under a microscope to look for the presence of typical *Mycoplasma* spp. colonies. Each specimen dilution inoculated in broth was plated on PPLO agar and re-incubated at 37 °C for 5–6 days at 10% CO_2 atmosphere. Plates were then examined for the presence of typical *Mycoplasma* spp. colonies.

4.2. Cloning

A single typical *Mycoplasma* spp. colony observed on agar plates was inoculated in PPLO broth and incubated at 37 °C for 5–6 days at 10% CO_2 atmosphere. Broth culture was then plated on PPLO agar to verify its purity.

4.3. Identification

0.3–0.4 ml of pure broth culture was inoculated in different broth media, each containing a different substance (glucose, arginine, tetrasodium aerobiosis, tetrasodium anaerobiosis, mannose and urease). After the usual incubation (37 °C for 5–6 days at 10% CO^2 atmosphere) colonies were identified at species level.

4.4. Statistical analysis

The effects of *Mycoplasma* spp. and *M. bovis* to determine lung lesions were evaluated through logistic regression. Lungs were classified with or without lesions and then considered as a binary response variable, while *Mycoplasma* spp. and *M. bovis* infections were considered as explanatory variables. All analyses were performed with R (R Development Core Team, 2011) setting $P < 0.05$ for significance.

5. Results

The majority of lesions seen in the calves could be classified as one of the following:

(1) Chronic catarrhal bronchopneumonia; characterized by reddish areas of multilobular consolidation, mostly distributed in cranial-ventral regions of the lungs, with abundant catarrhal and/or purulent bronchial exudate.
(2) Chronic bronchointerstitial pneumonia; characterized by multifocal grey-reddish areas of parenchymal consolidation and retraction, mostly distributed on cranial and medium pulmonary lobes, with no bronchial exudate.
(3) Acute-subacute bronchopneumonia; characterized by pulmonary congestion and multilobular dark red areas of parenchymal consolidation, with no bronchial exudate.

As shown in Table 1, 83 out of 224 (37%) lung tissue samples examined were positive at PCR test for *Mycoplasma* spp.; in 64 cases positive for *Mycoplasma* spp., we observed typical respiratory lesions. In particular, 25 cases were correlated to chronic catarrhal