Small Ruminant Research 153 (2017) 103–106

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Short communication

Mycoplasma pneumonia in small ruminants: A ten-year long retrospective survey

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ARTICLE INFO

Keywords:
Sheep
Goat
Pneumonia
Mycoplasma
Central Italy

ABSTRACT

Mycoplasma infections are commonly associated with respiratory diseases in farm animals. However, few data are currently available about the presence and the etiology of mycoplasma pneumonia in small ruminants in central Italy, a region particularly devoted to pastoralism. The present study aims to investigate retrospectively the causative agents of pneumonia in sheep and goats, a special emphasis being placed upon mycoplasmas. In total, respiratory pathogens were identified in 129 of 380 carcasses (32.36%). Mycoplasmas were detected in a high percentage of cases, alone (59 animals) or in combination with other pathogens (26 animals), *Mycoplasma arginini* being the most frequent species. No evidence of mycoplasma infection was demonstrated in 44 carcasses; in such animals, *Mannheimia haemolytica* and *Pasteurella multocida* were most commonly isolated. Overall, our results suggest that mycoplasma infections can contribute to a relevant portion of respiratory diseases in small ruminants and support the role of *Mycoplasma arginini* as a frequent cause of pneumonia in sheep. We consider that such data are of value to efficiently manage the health status of animal populations.

1. Introduction

Respiratory diseases represent a serious concern for the profitability and the welfare of farm animals (Nicholas et al., 2008). In small ruminants, pneumonia most commonly occurs in lambs and kids aged between 3–12 months, when maternal antibodies have waned. Pneumonia can affect individuals or groups and is often caused by a harmful combination of pathogenic microorganisms and environmental factors (Ruffin, 2001; Scott, 2011).

Mannheimia haemolytica can act as a primary or secondary pathogen and is considered to be the most important cause of bacterial pneumonia in sheep (Scott, 2011). By contrast, the role of mycoplasmas as pathogens of the lower respiratory tract is often overlooked (Ayling et al., 2004; Nicholas et al., 2008).

Mycoplasma pneumonia (also known as "atypical pneumonia" or "enzootic pneumonia") is a slowly progressive, chronic disease usually caused by *Mycoplasma ovipneumoniae*, although other *Mycoplasma* species, e.g. *M. arginini*, *M. mycoides* subsp. *capri*, *M. agalactiae*, may also contribute. Clinical signs range from subclinical to severe respiratory distress, depending on exacerbating concurrent factors, e.g. infection by *M. haemolytica*. In general, affected sheep show chronic, persistent, soft cough and ocular and nasal discharge. Most of the flock can be affected after several weeks, but the disease is rarely fatal and commonly goes undiagnosed (Nicholas et al., 2008; Ruffin, 2001). Gross lesions typically affect the cranioventral portion of both lungs and appear as sharply demarcated, red-to-greyish areas of consolidation (Nicholas et al., 2008).

To date, few data are available about the presence and the etiology of atypical pneumonia in small ruminants in Italy. Therefore, the aim of this retrospective survey is to add further knowledge about the causative agents of respiratory disease in sheep and goats, a special emphasis being placed upon mycoplasma pneumonia.

2. Materials and methods

2.1. Animals

The present investigation was carried out between 2004 and 2014 on small ruminant carcasses submitted for diagnostic purposes to the "*Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise*" (IZSAM, Teramo, Central Italy) laboratories. A total of 380 animals (319 sheep and 61 goats), which aged between 1 and 24 months and showed foci of pneumonia affecting the cranioventral portion of both lungs (Fig. 1), were included in this study.

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http://dx.doi.org/10.1016/j.smallrumres.2017.06.004
Received 28 February 2017; Received in revised form 23 May 2017; Accepted 1 June 2017
Available online 03 June 2017

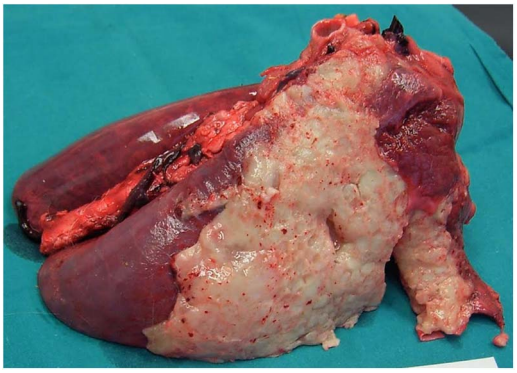

Fig. 1. Lamb. The cranioventral portion of the right lung is covered by a thick layer of fibrin. Bacteriological investigations yielded the isolation of *M. ovipneumoniae*, *M. arginini* and *M. haemolytica*.

2.2. Mycoplasma isolation

At necropsy, samples were collected from lymph nodes (mediastinal, trachea-bronchial lymph nodes) and diseased lung parenchyma, at the edge of foci of consolidation. Samples were about 1.5 cm^3 in size, were put with 10 ml of tryptose broth and homogenized in sterile bags. The homogenate was then centrifuged (1400 × g, for 15 min at 4 °C) and the supernatant filtered through a membrane with 0.45 µm pore size. Finally, 6–7 drops were cultured in CCPP-modified broth and plated on CCPP-modified agar medium (Anonymous, 2014). Broth and plates were incubated for one week at 37 °C with 5% CO_2 and examined daily for evidence of growth. Whenever *Mycoplasma* spp. isolation was suspected in CCPP modified broth, a subculture was carried out on a fresh CCPP-modified agar plate. Samples were considered negative when no evidence of growth was seen after one week of incubation.

2.3. Identification of mycoplasma

Until 2006, mycoplasmas were identified by the conventional "growth inhibition test" (Poveda and Nicholas, 1998). Thereafter, mycoplasmas' identification was carried out by biomolecular methods (polymerase chain reaction, PCR). More in detail, positive samples were initially tested by PCR specific for *M. arginini* (Volokhov et al., 2006), *M. putrefaciens* (Peyraud et al., 2003) and *M. ovipneumoniae*. In the latter case, primer sets were developed (5′-TGG GGA AAC CCA ACC TAG CA-3′ and 5′-ACG GTT TGG GCT CCT CCC ATC-3′) based on the intergenic 16S-23S rRNA spacer region of *M.ovipneumoniae* (NCBI Reference Sequence: NZ_JAKV01000001.1).

A PCR mixture was prepared, containing 25 µl Top-taq Master mix kit Qiagen® 2X, 18 µl nuclease free water, 1 µl of each primer (concentration of each primer = 50 pmol/µl) and 5 µl of target DNA. After pre-heating to 94 °C for 5 min, 40 cycles were performed, each consisting of a 94 °C 30-s denaturation, a 56 °C 30-s annealing and a 72 °C 30-s extension. The last cycle ended with a 72 °C 7-min final extension. Amplicons were visualized by agarose gel electrophoresis.

Each sample was further tested by PCR specific for *M. agalactiae* (Tola et al., 1996), *M. mycoides* subsp. *capri* (Nicholas and Bashiruddin, 1996) and *M. alkalescens* (Kobayashi et al., 1998)

2.4. Bacteriological investigations

Lung samples were submitted to conventional bacteriological methods. To this aim, samples were plated on sheep blood agar, MacConkey and mannitol salt agar. Plates were cultured at 37 °C with 5% CO_2 for 72 h; bacterial colonies were identified by VITEK® MS and API systems.

3. Results

3.1. Sheep

Data are graphically summarized in Figs. 2–4 . The presence of only mycoplasmas, in absence of other pathogens, was demonstrated in 55 ovines, *M. arginini* being the most commonly detected species. In particular, *M. arginini* was detected alone in 29 cases and associated with other *Mycoplasma* species in 11 animals. *M. ovipneumoniae* was the only detectable pathogen in 2 cases, while it was demonstrated in combination with other *Mycoplasma* species in 9 subjects. Other mycoplasmas were only occasionally isolated.

Mycoplasma infections were often combined with other bacteria, the most frequent combinations being *M. arginini/M. ovipneumoniae/M. haemolytica* (n = 5) and *M. arginini/Pasteurella multocida* (n = 5).

In 43 ovines, bacteriological investigations yielded positive results, with no evidence of mycoplasma infection; in those animals, *M. haemolytica* (n = 23) and *P. multocida* (n = 11) were the most frequently

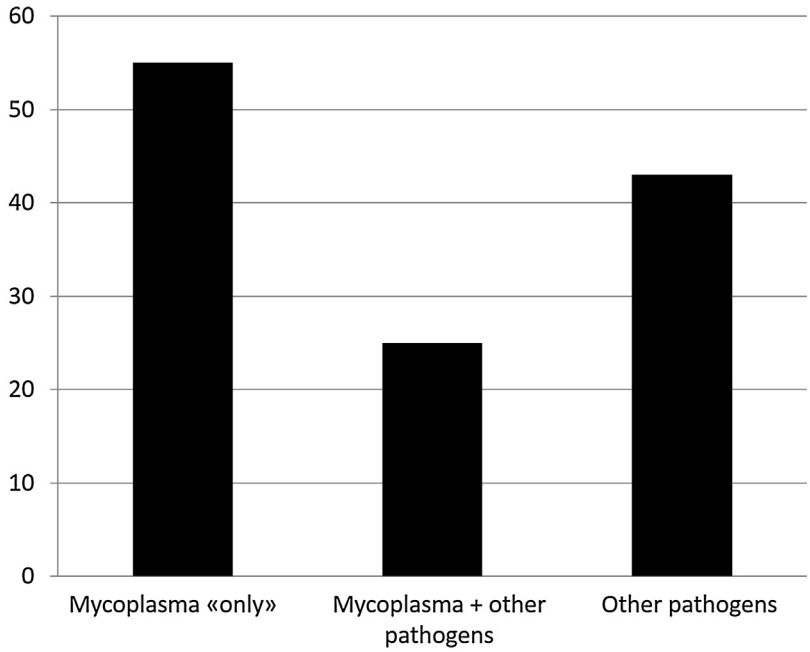

Fig. 2. Summary of microbiological results. Overall, pathogenic microorganisms were identified in 123/380 (32.36%) carcasses. Mycoplasma infections were demonstrated in about 2/3 of positive cases. In 55 animals, only *Mycoplasma* – of different species, alone or combined – were detected. In 25 ovines, co-infections by *Mycoplasma* and other pathogens (e.g. *M. haemolytica*, *P. multocida* etc) were demonstrated, while in 43 ovines no evidence of *Mycoplasma* infection was shown.