Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Veterinary Medicine and Animal Science
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Association of *Mycoplasma ovipneumoniae* Infection with Respiratory Disease in Swedish Sheep

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Mycoplasma ovipneumoniae som orsak till lunginflammation hos svenska får

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SUMMARY

The Swedish lamb production is steadily increasing and pneumonia is one of the diseases causing the greatest income losses. Pneumonia and pleuritis are two of the most common post-mortem inspection findings registered at routine slaughter amongst both sheep and lambs, and pneumonia is the most common finding at necropsied adult sheep that have been found dead.

It is essential to know which infectious agent causing the pneumonia in order to design an ideal treatment protocol for the flock. But the diagnostics of live animals is problematic and limited both by cost and by the fact that several of the bacteria is part of the commensal flora of the upper respiratory tract of healthy sheep, which can make the results unreliable. *Mycoplasma ovipneumoniae* is one of the agents associated with respiratory disease and is a part of the aetiology of mycoplasmosis, also called atypical pneumonia.

Penicillin is generally the first choice of treatment of suspected pneumonia but *Mycoplasma* spp. are naturally resistant against penicillin and therefore require antibiotics with a broader spectrum whether it should be treated. To reduce unnecessary use of broad-spectrum antibiotics it is necessary to increase knowledge about pneumonia in sheep and to develop the diagnostics.

If there were a way to determine the cause of the lesions already at the post-mortem inspection at slaughter you could get a foundation for a treatment strategy. Therefore, the aim of this study has been to examine whether a specific agent can be correlated to a specific gross pathological or histopathological appearance, as well as studying the prevalence of *Mycoplasma ovipneumoniae* and other bacterial agents in lung lesions found at routine slaughter in Sweden.

A total of 44 lungs with pneumonic lesions were collected from two slaughterhouses during the period of September-October. The lungs were examined macroscopically and histologically, photographed and samples were taken for a microbiological investigation including an aerobic bacterial culture and a Mycoplasma PCR. Three of the lungs were excluded from the project due to a deviant gross or histological appearance. *Mycoplasma ovipneumoniae* were isolated in 76% of the remaining lungs and *Mannheimia haemolytica* in 63%, always in combination with *Mycoplasma* spp.

Mycoplasma ovipneumoniae did not correlate with any of the histological findings but could be correlated to a specific gross appearance.

The study is financed by the Swedish SvarmPat program.

SAMMANFATTNING

Sveriges lammproduktion ökar stadigt och lunginflammation är en av de sjukdomar som orsakar störst inkomstförluster. Lunginflammation och pleurit är två av de vanligaste fynden som registreras vid slaktkroppsbesiktning på slakterier, och lunginflammation är den vanligaste diagnosen som ställs vid obduktion av får som har dött plötsligt.

För att utforma ett optimalt behandlingsprotokoll för besättningen är det essentiellt att veta vilket agens som orsakar lunginflammationen. Men diagnostiken på levande djur är problematisk och begränsas både av kostnad och av att många av bakterierna även kan förekomma normalt i näshålan hos friska får vilket gör resultaten osäkra. *Mycoplasma ovipneumoniae* är ett av de agens som hittas i samband med lunginflammation och orsakar det som i Sverige kallas för pälsfårshosta.

Penicillin är generellt förstahandsvalet för behandling av misstänkt lunginflammation men då *Mycoplasma* spp. är naturligt resistenta mot penicillin krävs ett antibiotikum med bredare spektrum om den ska behandlas. För att minska onödig användning av bredspektrumantibiotika är det nödvändigt att öka kunskapen kring vilka agens som brukar orsaka lunginflammation samt att utveckla diagnostiken.

Den här studien undersöker möjligheterna att utveckla ett system för att sortera ut besättningarna där hostan orsakas av *Mycoplasma ovipneumoniae* från besättningarna där orsaken till hostan är en annan. Om det vore möjligt att vid slaktkroppsbesiktningen utröna orsaken till lunginflammationen får man en grund för en bra behandlingsstrategi i den aktuella besättningen. Därför är målet med denna studie att undersöka om ett specifikt agens kan korreleras mot ett specifikt makroskopiskt eller histologiskt utseende, samt att även undersöka frekvensen av *Mycoplasma ovipneumoniae* i lunglesioner funna vid normalslakt i Sverige.

I denna studie som finansierats av svenska SvarmPat har totalt 44 lungor med lunginflammation samlats in från två slakterier i Uppland. Lungorna undersöktes makroskopiskt och histologiskt, fotograferades och ett prov togs för aerob bakterieodling och mycoplasma-PCR. Tre av lungorna uteslöts från projektet på grund av deras avvikande makroskopiskt eller histologiskt utseende. Bland övriga isolerades *Mycoplasma ovipneumoniae* från 76% av lungorna och *Mannheimia haemolytica* från 63% av lungorna, alltid i kombination med *Mycoplasma* spp. *Mycoplasma ovipneumoniae* kunde korreleras med ett makroskopiskt utseende men gick inte att korrelera till något specifikt av de frekvent förekommande histologiska fynden.

CONTENT

INTRODUCTION

The Swedish lamb production is steadily increasing, both in the number of animals and in the number of farmers (Jordbruksverkets statistikdatabas). Pneumonia is one of the diseases amongst farm animals that causes the greatest income losses. (Jensen *et al.*, 2008)

According to Farm and Animal Health and their database of post-mortem inspection findings at routine slaughter, lambs in Sweden are over all healthy but pneumonia and pleuritis are two of the most common findings. Pneumonia was the second most common finding in lambs 2015, with *Dicrocoelium dentriticum*, the lancet liver fluke, as the most common finding. The prevalence of pneumonia as an inspection finding at routine slaughter has been increasing over the last few years, both amongst lambs and adult sheep (Fig. 1).

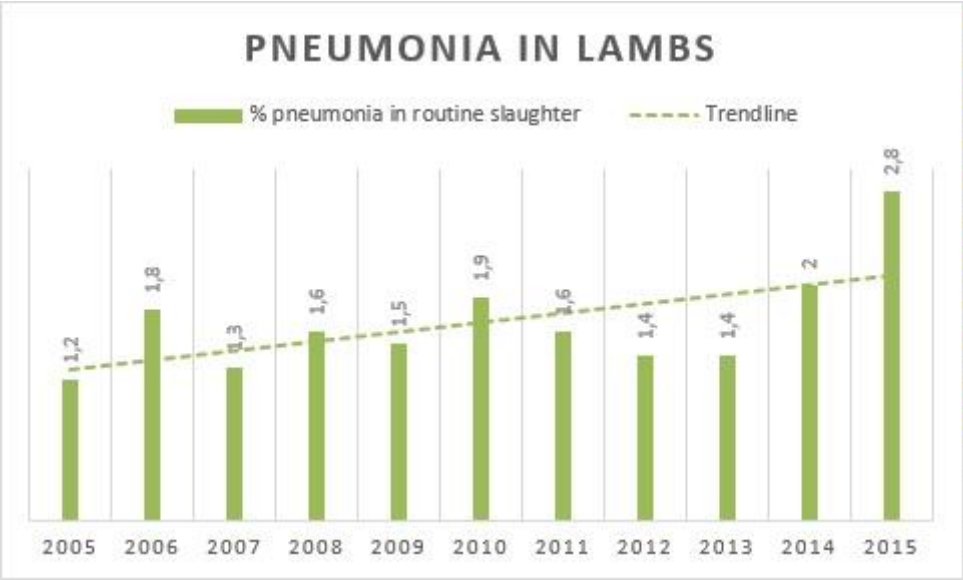

Fig. 1. *The prevalence of pneumonia as an inspection finding at routine slaughter amongst lambs in Sweden. (The database of post-mortem findings at routine slaughter of Farm and Animal Health)*

Pneumonia is also the most common finding in adult sheep at necropsies that is performed when the animals is found suddenly dead. In 2013, Farm and Animal Health financed 2200 necropsies on adult sheep. The results showed that 20% of the sheep had pneumonia, with meningitis as the second most common cause of death at 11%. Amongst lambs in the age between 2-12 months, pneumonia is also a common cause of death with almost as many findings as enteritis which is the most common finding (Lundström, 2015).

The aetiology of pneumonia in sheep is multifactorial and complex. Important pathological agents include bacteria, mycoplasmas, parasites and viruses like the maedi visna-virus. Pathological agents are often described separately but many agents can also occur simultaneously in one animal. That makes respiratory disease in sheep a complicated complex, in which everything is not yet known today. The two main groups of bacterial respiratory disease are called pasteurellosis/mannheimiosis or mycoplasmosis depending on the main cause of the disease (Hammarberg, 2014).

The true prevalence of mycoplasmosis as a cause of pneumonia in the Swedish sheep population is not well known. A study was performed during 2010-2012 where they tested the prevalence of *Mycoplasma ovipneumoniae* amongst sheep with pneumonic lesions found at necropsy. *Mycoplasma ovipneumoniae* were detected in 5/15 samples 2010, 18/56 in 2011 and 12/31 in 2012. That is about one third of the tested pneumonic lungs (Unnerstad, H., SVA, 25/10/2016).

The first choice of treatment for bacterial pneumonia in sheep is penicillin. As of today, penicillin resistance has not been confirmedamongst *Pasteurella* spp. or *Mannheimia* spp. from sheep in Sweden

(Unnerstad, H., SVA, 09/11/2016). Bacteria from the *Mycoplasma* genus lack cell wall which give them a natural resistance to penicillin. Therefore, tetracycline is the first choice of treatment of *Mycoplasma ovipneumoniae*. The difficulty in determining bacterial aetiology of pneumonia *in vivo* increases the risk for unnecessary use of broad-spectrum antibiotics against penicillin sensitive bacteria.

Currently, there is a need to improve the knowledge and to develop field diagnostics in order to make it possible to create treatment strategies in cases where it is difficult to determine if antibiotics is necessary, and in that case which active substance of antibiotics should be used.

This project examines the possibility to develop a system to sort out the flocks where the cause of the cough is *Mycoplasma ovipneumoniae* from flocks where the cause is another based on postmortem findings at slaughter. If there were a way to determine the cause of the lesions already at the post-mortem inspection at slaughter you could get a foundation for a treatment strategy. Therefore, the aim of this study has been to examine if a specific agent can be correlated to a specific gross pathological or histological appearance, as well as studying the prevalence of *Mycoplasma ovipneumoniae* and other bacterial agents in lung lesions found at routine slaughter in Sweden.

The project is a part of the SvarmPat program.

LITERATURE REVIEW

SvarmPat

SvarmPat is a cooperation program against antibiotic resistance. SVARM stands for Swedish Veterinary Antimicrobial Resistance Monitoring. SvarmPat is run in collaboration between Farm and Animal Health and The Swedish National Veterinary Institute (SVA), and is financed by the Swedish Board of Agriculture. Farm and Animal Health is an advisory company with the aim of maintaining a high level of health amongst farm animals.

The aim of the SvarmPat program is to monitor and work against the development of antimicrobial resistance in bacteria causing disease in pigs, cattle, sheep or poultry. Several studies are done within the program with the aim to strengthen our knowledge in the area of antibiotic resistance (SVA, 2016a).

Pneumonia in sheep

Respiratory disease in small ruminants is responsible for substantial financial losses worldwide. In addition to death and sickness, reduced feed efficiency, slaughter condemnations and treatment measures contribute substantially to the losses (Ayling & Nicholas, 2007). Pneumonia counts as one of the greater health issues also in the Swedish sheep population (SVA, 2016). The symptoms vary in severity between subtle health impact with increased respiratory rate and impaired growth to sepsis and sudden death. Initial symptoms are occasional coughs, nasal discharge, fever and inappetence (Nicholas *et al.,* 2008). Furthermore, pneumonia is a differential diagnosis to hypocalcaemia, pregnancy toxemia and cerebrocortical necrosis (CCN) amongst ewes in late pregnancy when the main symptom is sternal recumbency and lethargy (SVA, 2016b).

Pneumonia can be classified on the basis of various criteria, for example aetiology (Pasteurella Pneumonia, Viral pneumonia, Histophilosis pneumonia etc.), type of exudate (suppurative, fibrinous, granulomatous pneumonias), epidemiology (enzootic pneumonia, contagious bovine pleuropneumonia etc.) and distribution (lobar, lobular, diffuse, interstitial, focal etc.). It is not unusual to combine different classifications to reach the clearest result (Jensen *et al*, 2008).

The aetiology of pneumonia amongst sheep is multifactorial and complex. Important pathological agents include bacteria, mycoplasmas, parasites and viruses like the maedi visna-virus. Several authors both in Sweden and abroad classify the two main groups of bacterial pneumonia as mannheimiosis/pasteurellosis and mycoplasmosis, depending on which agent is causing the disease. It is common that several agents occur simultaneously in one animal, nonetheless, pathological agents are often described separately. That make respiratory disease in sheep a complicated complex, in which there are still unknown factors (Donachie, 2007; Ayling & Nicholas, 2007; Hammarberg, 2014).

Severel viral agents have been isolated in sheep with respiratory disease, for example; para-influenza type 3 virus (PI3), adenoviruses and the respiratory syncytial virus (RSV) (Sharp & Nettleton, 2007; de Verdier, K., SVA, 18/10/2016). This study will focus on pneumonia with a bacterial aetiology.

Pneumonic Mannheimiosis/Pasteurellosis

Pneumonic mannheimiosis/pasteurellosis is caused either by *Mannheimia haemolytica, Bibersteinia trehalosi* or *Pasteurella multocida* (Hammarberg, 2014). All three of them are derived from the same family, *Pasteurellaceae.*

An old species name of *Mannheimia haemolytica* is *Pasteurella haemolytica*, biotype A. (Swedish University of Agricultural Sciences/VetBact, 2015). *Bibersteinia trehalosi* was earlier called *Pasteurella haemolytica* biotype T or *Pasteurella trehalosi* (Swedish University of Agricultural Sciences/VetBact, 2013).

Nicholas *et al.* (2008) claims it is the most common cause of pneumonia in sheep, and that it occurs worldwide.

Mannheimia haemolytica is a normal inhabitant of the upper respiratory tract of sheep. In healthy flocks there is considerable sheep-to-sheep variation in the biotype and serotype present in the nasopharynx. There are also cyclical changes in carrier rate with time and changes in the predominant serotypes that are present. The prevalence of carriers is higher in flocks that are experiencing disease when compared to healthy flocks. The bacteria can also be present in the environment, with a prolonged survival in cool and wet conditions (Radostits *et al.,* 2007).

In some of the cases, no obvious clinical signs precede death and the animal is found deceased. When clinical signs are present they may include dullness, anorexia, pyrexia of greater than 40,6 degrees Celsius, serous nasal discharges and hyperpnea/dyspnea. Clinical signs may be less obvious in subacute or chronic cases, rather than in the acute cases (Donachie, 2007).

Both *Mannheimia haemolytica* and *Bibersteinia trehalosi* causes septicemia and sudden death in young lambs at pasture. In addition, *Mannheimia haemolytica* can also cause pneumonia in sheep of all ages during the autumn and winter months (Hammarberg, 2014).

Beyond three months of age most cases are pneumonic although sudden deaths with septicemia rather than pneumonia may still occur. Morbidity and mortality vary (Donachie, 2007). Stress, subclinical infections by parainfluenza virus type 3 or mycoplasmas may predispose animals to outbreaks of pneumonia (Nicholas *et al.,* 2008). Necrotic cells and proteinaceous fluid in the lungs due to a virus infection is thought to create an ideal micro-environment, favoring bacterial growth by interfering with the mucociliary clearance mechanisms and by depressing the capacity of resident lung macrophages to take up and kill bacteria (Donachie, 2007).

Necropsy findings in sheep that have died from peracute pneumonic pasteurellosis can include a greenish gelatinous exudate over the pericardium and large quantities of straw-coloured pleural exudate. The lungs are enlarged, edematous and haemorrhagic. Consolidation of the lungs is present in less acute cases (Radostits *et al.,* 2007).

Mannheimia haemolytica is a primary pathogen in very young lambs whilst older lambs are more resistant and predisposing factors are required for the development of the disease. *Mannheimia haemolytica* is a secondary invader, and a cause of death, in chronic enzootic pneumonia in sheep associated with *Mycoplasma ovipneumoniae. Mannheimia haemolytica* produces a leukotoxin considered to be an important virulence factor and promotes bacterial proliferation by killing ruminant neutrophils and pulmonary macrophages (Radostits *et al.,* 2007).

Mycoplasmosis

The disease is most often called mycoplasmosis after *Mycoplasma ovipneumoniae,* but is a disease with a multi-factorial aetiology were many agents can occur simultaneously (Radostits *et al.*, 2007; *Hammarberg,* 2014). Mycoplasmosis is also known as atypical pneumonia, chronic enzootic pneumonia or chronic non-progressive pneumonia. In some literature atypical pneumonia is even explained as the combined infection of *Mycoplasma ovipneumoniae* and *Mannheimia haemolytica.*

A Swedish name for the disease is “pälsfårshosta”, named after the breed where the disease first was detected in Sweden. It is now well known that the disease isn’t restricted to only one breed.

Fatal Pneumonia in Bighorn Sheep

During 1979-1980 acute fibrinopurulent bronchopneumonia resulted in high mortality or total loss of herds of bighorn sheep (*Ovis canadensis)* in California and Washington. Lungs were bilaterally reddened, wet and consolidated in an cranioventral distribution and fibrinous pleural adhesions and pulmonary abscesses were also noted. Contact with domestic sheep occurred shortly before outbreak of disease in all cases. Fatal acute pneumonia in otherwise healthy individuals within 26 days after

introduction of domestic sheep suggests that a transmission of a pathogen from the domestics occurred. *Pasteurella* spp. were isolated from several individuals (Foreyt & Jessup, 1982).

Besser *et al.* (2008) associated *Mycoplasma ovipneumoniae* with the outbreaks of bronchopneumonia in bighorn sheep. Using PCR, they found *Mycoplasma ovipneumoniae* as a consistent finding in pneumonic bighorn sheep, both in their recent study and in preserved lung tissue material from old bighorn sheep specimens.

The same research group decided to test the hypothesis that *Mycoplasma ovipneumoniae* is an important agent of the bighorn sheep pneumonia and tried to put four bighorn sheep in contact with domestic sheep that were proven free from *Mycoplasma ovipneumoniae.* One of the bighorn sheep died in fatal pneumonia 90 days after contact, but the other three remained healthy. They suggest that this survival rate supports the hypothesis that *Mycoplasma ovipneumoniae* is an important pathogen of pneumonia in bighorn sheep (Besser *et al.,* 2012).

Moreover, Dassanayake *et al.* (2010) performed a small study on four bighorn sheep. Two of the sheep received nasopharyngeal washings with *Mycoplasma ovipneumoniae* from domestic sheep, and the other two bighorn sheep were inoculated intranasally with *Mycoplasma ovipneumoniae.* All of the sheep developed clinical symptoms and one of them died at day 47. That sheep carried *Mannheimia haemolytica* in the nasopharynx since before. The remaining three were inoculated intranasally with *Mannheimia haemolytica* on day 70 and they all developed pneumonia and died within 1-5 days after inoculation. All four of them showed signs of bronchopneumonia on the post-mortem investigation and both *Mannheimia haemolytica* and *Mycoplasma ovipneumoniae* were isolated from the lungs. The author suggests that *Mycoplasma ovipneumoniae* alone may not cause fatal pneumonia in bighorn sheep, but that it may predispose to fatal pneumonia due to secondary *Mannheimia haemolytica* infection.

Besser *et al.* (2013) reviewed the evidence for each of the candidate primary pathogens causing bighorn sheep pneumonia. They suggest that healthy bighorn sheep populations are naïve to *Mycoplasma ovipneumoniae,* and that its introduction to susceptible populations result in pneumonia were several bacterial agents occur and that it is followed by a chronic infection in recovered adults.

The possibility of reducing the risk of *Mycoplasma ovipneumoniae* infection in bighorn sheep have been discussed. Ziegler *et al.* (2014) approached this objective by trying to control the pathogen in its reservoir hosts - the domestic sheep. The safety and immunogenicity of *Mycoplasma ovipneumoniae* were examined in three experimental immunization protocols. In one protocol with a relatively large antigenic mass combined with an adjuvant, the ewes developed significant serum antibody responses and *Mycoplasma ovipneumoniae* inhibition activity, and these responses were passively transferred to their lambs.

Pneumonia in Domestic sheep

There is disagreement about the role *Mycoplasma ovipneumoniae* plays as a causative agent in pneumonia.

Mycoplasma ovipneumoniae can be found in a high prevalence in the respiratory tract of both healthy sheep as well as in sheep with respiratory disease. Therefore, it has been suggested that it may be a part of the normal commensal flora or that during times of stress, a subclinical infection of *Mycoplasma ovipneumoniae* may predispose sheep to a more serious respiratory disease (Brogden *et al.,* 1988; Alley *et al.,* 1999).

Davies *et al.* (1981) suggests that *Mycoplasma ovipneumoniae* is neither an important primary or secondary lung pathogen, based on a study were several groups of specific-pathogen-free lambs were inoculated with para-influenza virus type 3, *Mannheimia haemolytica* and *Mycoplasma ovipneumoniae* in different combinations. None of the lambs inoculated with *Mycoplasma ovipneumoniae* alone or in combination with para-influenza virus developed pulmonary lesions.

However, eight out of nine lambs developed bronchopneumonia in the group inoculated with para-influenza virus followed by *Mannheimia haemolytica*. Sheehan *et al.* (2007) did an aetiopathological study of chronic bronchopneumonia in lambs where they collected thirty sets of sheep lungs with grossly visible pneumonic lesions. The lesions were associated with *Mycoplasma ovipneumoniae*, identified by either culture or immunohistochemistry, in a total of 90% of the cases. These results may contradict the suggestions of Davies *et al.* (1981).

Some *in vitro* studies shows that *Mycoplasma ovipneumoniae* affect parts of the lungs defenses. It has the ability to attach to alveolar macrophages without inducing phagocytosis, but it does stimulate to cellular mitosis. If then mycoplasma specific antibodies are introduced they stimulate to an effective phagocytosis and extensive spreading of alveolar macrophages over the coverslip (Al-Kaissi & Alley, 1983). Another *in vitro* experiment showed that *Mycoplasma ovipneumoniae* can inhibit ciliar activity with hydrogen peroxide production. This could contribute to the role *Mycoplasma ovipneumoniae* may play in sheep respiratory disease (Niang *et al.*, 1998).

Radostits *et al.*, (2007) describes the disease as common, low pathogenic with a multi-factorial etiology including *Mycoplasma ovipneumoniae,* and different viruses. The disease affects lambs under 12 months of age and can cause consolidation of the cranioventral lung lobes and pleuritis. *Mannheimia haemolytica* is a common secondary infection and may lead to a more severe respiratory disease. The cause of the disease is not well understood, and the authors suggest it is due to its non-fatal character which lead to incomplete examination of early cases. Most of those submitted to postmortem examination are difficult to make conclusions from due to overgrowth of secondary pathogens.

Mycoplasma pneumonia is well recognized in both Australia and New Zeeland where it is known as ”summer pneumonia” because of the seasonal difference in the prevalence of the disease. It may be a difference in virulence among isolates. The outbreaks usually affect animals in their first year of life with high morbidity and low mortality, poor growth rates and exercise intolerance. Clinical signs vary from increased respiratory rates to mortality with acute fibrinous pneumonia, consolidated lesions, pulmonary abscesses and pleuritis, depending on aggravating circumstances. The main clinical signs are chronic, persistent cough which may lead to rectal prolapses and mucopurulent nasal discharge (Ayling & Nicholas, 2007).

Ayling & Nicholas (2007) suggest that sheep may act as a major source of infection to lambs, since *Mycoplasma* spp. frequently occur in the upper respiratory tract in healthy sheep. Lambs are thought to be infected within the first days of life but the disease progress slowly and clinical signs often occurs with a secondary infection from 5 to 10 weeks of age. Outbreaks can occur when lambs from different flocks are housed together and may be a result of mixing uninfected with infected lambs or the effect of encountering different strains of *Mycoplasma ovipneumoniae.*

In an examination of the aetiology of a pneumonia outbreak in Iran, *Mycoplasma* spp. was isolated from all examined sheep. No *Mycoplasma* spp. could be isolated from sheep without clinical signs. The flock consisted of 180 sheep in a crowded area with poor ventilation and a dusty environment. The prominent clinical signs included fever, coughing, respiratory distress, nasal discharge, hot and swollen joints, lameness and depression. Complete necropsies were performed on 11 sheep that died. The authors suggest that *Mycoplasma* spp. can show a septicemic nature even if it in general induces a chronic disease with low mortality. Histopathological findings that supported this included vasculitis and thrombosis (Mohkber Dezfouli *et al.*, 2011). Stress, poor air quality and adverse weather conditions can contribute to the disease (Caswell & Williams, 2015), which may explain the severity in this outbreak.

Another example where adverse environment conditions may have worsened clinical signs of mycoplasmosis is a growing number of outbreaks of pneumonia in flocks in the United Kingdom since 2001. The flocks were fully vaccinated with pasteurella vaccines and *Mycoplasma ovipneumoniae*

were isolated. For comparison, lambs in clinically healthy flocks of the same size and management type were also examined, and *Mycoplasma ovipneumoniae* was not detected in the same extent. The increase in isolation from pneumonic flocks since 2001 may be related to overcrowding following restrictions of movement imposed during the foot-and-mouth disease outbreaks (McAuliffe *et al.*, 2003).

Diagnosis

To make an ideal treatment protocol it is vital to know the aetiology of the respiratory disease in the particular sheep flock. That is, however unrealistic in most general veterinary practice situations since several causative agents give rise to similar clinical signs. Additionally, financial restrictions often limit available laboratory resources (Scott, 2011).

Auscultation

Auscultation of the chest is a fundamental part of the clinical examination of sheep. Abnormal findings can include increased audibility of normal lung sounds, tachypnea or abnormal sounds like clicking, popping, bubbling, crackling or wheezes.

Scott (2010) auscultated both normal sheep and sheep diagnosed with ovine pulmonary adenocarcinoma with ultrasonographic examination. The distribution of lesions was then confirmed through necropsy. Rumen contraction sounds are often superimposed upon lung sounds and increased audibility of lung sounds was common due to exercise or stress and not only due to pain, toxemia or fever. Moderate to severe crackles were identified in severe cases but did not correspond well to distribution of lesions. Auscultation could not detect focal pleural abscesses. Auscultation alone is not sufficient enough to determine the presence of all superficial lung pathology nor accurately define its distribution.

Laboratory Blood Test

An inflammatory response to bacterial infection can make a change in the leukogram, the haptoglobin, fibrinogen and serum protein concentrations, but these changes are not specific for respiratory disease (Scott, 2011).

Nasal Swabs

Fjällström (2008) tested 56 sheep with both an ordinary nasal swab and a nasal swab with a protective tube that was designed to prevent contamination from the most external parts of the nasal cavity. The tested animals were from flocks that had a problem with cough and other signs of respiratory disease. A total of 69% tests taken with the protective tube showed a purer culture than the ones taken with an ordinary nasal swab. Despite that, all of the tests showed a mixed population of bacteria.

Donachie (2007) consider nasal swabs to be of no diagnostic significance, and refers to Gilmour & Gilmour (1985) who claims that nasal swabs only indicate the presence of the bacteria in the nasopharynx and not that the sheep are affected with disease. Isolation in culture of large numbers from exudate or from the cut surfaces of lung lesions confirms acute pneumonic pasteurellosis (10^6 or more colony-forming units, CFU, per g of lung). In subacute and chronic cases, 10^3-10^5 CFU/g would be expected. Samples should be collected from untreated cases (Donachie, 2007).

Ultrasonography

Ultrasonography is an inexpensive and non-invasive method to further examine possible lesions in the chest. Scott & Sargison (2010) used a modern portable ultrasound machine together with a 5.0 MHz sector transducer and presented good images of fibrinous pleuritis, consolidated areas of the lung parenchyma, pleural abscesses and other lesions. This offers the possibility to establish a crude diagnosis in most cases of respiratory disease.

Radiographic examination

Pathologic changes associated with aerosol infection commonly involve the cranioventral lung field, where radiographic examination is restricted by the thoracic limbs and associated musculature (Scott, 2011).

Bronchoalveolar Lavage

Sheehan *et al.* (2005) tested a transtracheal bronchoalveolar lavage technique for diagnosing respiratory disease in sheep under field conditions. Seventy-six sheep were divided into three groups, normal sheep, sheep with clinical signs of respiratory disease and housed sheep without a history of respiratory disease.

The sheep was restrained in a standing position with its neck slightly extended and an area over the trachea was clipped and disinfected. It was then infiltrated with lidocaine hydrochloride. An intravenous catheter was introduced between two tracheal rings. When it was in place, an extension was introduced through the catheter and advanced down the trachea until resistance was encountered. Thirty milliliters of sterile isotonic saline were infused and usually well tolerated by the animal. The fluid was withdrawn after 5 seconds. No aftercare was necessary (Sheehan *et al.*, 2005).

The detection of *Mannheimia haemolytica* and *Mycoplasma ovipneumoniae* or parainfluenza type 3 virus and bovine respiratory syncytial virus antigen in the lavage samples was closely correlated with clinical disease (Sheehan *et al.*, 2005).

Treatment

Pasteurellosis

To make an ideal treatment protocol it is essential to know the aetiology of the respiratory disease in the particular sheep flock (Scott, 2011).

Scott (2011) frequently mentioned the importance of a rapid detection of sick sheep which is necessary for a good treatment response. The author mentions oxytetracycline as the antibiotic treatment most commonly selected.

In Sweden, the first choice of treatment is penicillin and the second choice of treatment is tetracycline. Penicillin resistance amongst *Pasteurella* spp. or *Mannheimia* spp. from sheep in Sweden has not been confirmed (Unnerstad, H., SVA, 09/11/2016).

The severity of clinical pneumonic pasteurellosis is correlated with episodes of endotoxemia, bacteremia and elevated eicosanoid concentrations (Hodgson *et al.*, 2003). Therefore, it is recommended to combine antibiotic treatment with a Nonsteroidal Anti-Inflammatory Drug (NSAID) preparation, together with other supportive therapy (Scott, 2011).

Optimal control against pasteurellosis is achieved by vaccination. However, there are circumstances in which the use of antibiotics is useful, for instance in outbreaks in lambs during the period when passive immunity from colostrum has waned and active immunity is being generated through vaccination (Donachie, 2007). Presently there are vaccines available that can be used in the control of both pasteurellosis and clostridial diseases.

Mycoplasmosis

Treatment is generally not necessary because clinical signs are often mild (Scott, 2011).

Treatment with antibiotics effective against mycoplasmas often give immediate recovery, but when *Mycoplasma ovipneumoniae* is involved, the animals may quickly relapse and require further treatments (Ayling & Nicholas, 2007).

It would probably be a successful treatment strategy to be able to also control *Mycoplasma ovipneumoniae* infections through immunization. However, the difficulty in developing protective vaccines to control *Mycoplasma hyopneumoniae,* the etiologic agent of porcine enzootic pneumonia,

illustrates the possible challenge of a future development of an effective *Mycoplasma ovipneumoniae* vaccine (Ziegler *et al.,* 2014).

The outbreaks of pneumonia in flocks vaccinated against pasteurellosis in the United Kingdom suggests that consideration should be given to incorporating a mixed selection of *Mycoplasma ovipneumoniae* strains into these vaccines. (McAuliffe *et al.,* 2003; Ayling & Nicholas, 2007)

Al-Kaissi & Alley (1983) demonstrated in an *in vitro* experiment that if sheep attained high titres of antibodies they may be able to deal quickly and effective with a *Mycoplasma ovipneumoniae* infection. To produce detectable serum antibody responses, there is a need for a relatively large antigenic mass. Future studies will be required to examine the ability of subcutaneous immunization to elicit mucosal antibodies (Ziegler, *et al.* 2014).

Lower stocking densities and improved ventilation are important in preventing and reducing the spread of respiratory disease (Ayling & Nicholas, 2007). The airspace should not be shared with older sheep that are carriers of causative agents. Furthermore, newly purchased lambs should be isolated from the homebred flock (Scott, 2011).

Histology

General

The trachea bifurcates into bronchi, which enter the lungs and then branch extensively. The lungs are covered by visceral pleura which is formed by connective tissue and some smooth muscle. A framework of connective tissue, rich in elastic fibers, form the interior of the lungs. It supports the bronchi and divides the lungs into lobules. A ciliated, stratified, columnar epithelium with goblet cells lines the bronchi. Smooth muscle and plates of hyaline cartilage surrounds the bronchi and lamina propria. The height of the epithelium diminishes when the bronchi get smaller in diameter (Bacha *et al.,* 2012).

Bronchioles lack cartilage. They are outlined by cuboidal epithelium that is ciliated proximally but becomes unciliated distally. The mucosa of the bronchioles is folded, unless the lungs were inflated in the moment of fixation. Terminal bronchioles divides into respiratory bronchioles. The respiratory bronchioles are lined by a cuboidal epithelium which becomes more flattened distally (Bacha *et al.,* 2012).

The respiratory bronchioles divide into alveolar ducts which have thin walls are constructed entirely of alveoli. Each alveolar duct branches into three or more alveolar sacs. Alveoli is lined by thin squamous epithelial cells (type I pneumocytes) and some type II pneumocytes, which produce surfactant (Bacha *et al.,* 2012).

Mycoplasmosis

Caswell & Williams (2015) describes chronic bronchopneumonia as a disease in lambs that can be caused by multiple agents like *Mannheimia haemolytica*, para-influenza type 3 virus, respiratory syncytial virus and where *Mycoplasma ovipneumoniae* also plays an important causal roll. The disease is characterized by lymphoid hyperplasia around airways, neutrophil accumulation within airspaces, hyperplasia of bronchiolar and alveolar epithelium, bronchiolar mucous metaplasia and a unique lesion of hyaline scars. Hyaline scars consist of nodular masses of eosinophilic matrix and fibroblasts within the wall of bronchioles that bluntly compress the lumen.

Sheehan *et al*., 2007, observed the same pulmonary lesions mentioned above but supplemented with the following interstitial lesions; leukocyte infiltration of alveolar septa, type II pneumocyte hyperplasia, interstitial fibrosis and atelectasis. However, the presence of *Mycoplasma ovipneumoniae* organisms or antigen did not consistently correlate with any particular histopathological changes.

Lung defenses

The lungs are susceptible to pathogens because of their inevitable exposure to the environment. Inhaled air contains immense quantities of microorganisms, foreign material and droplets aspirated from the non-sterile upper respiratory tract containing opportunistic pathogens. Because of the rich blood flow through the lungs, increasing numbers of non-commensal organisms in the alveoli carry a risk of bacteremia. The lungs have a number of defense systems to prevent this from happening (Caswell & Williams, 2015).

The major mechanisms for clearing particles trapped in the airways is coughing or clearance by ciliary movement. A mucus layer that contains sticky glycoproteins lines the airways and trap particles. The mucus is later transported to the pharynx where it is swallowed. The mucus also contains several types of innate antimicrobial factors with different operating mechanisms such as opsonizing bacteria or blocking the attachment of bacteria to mucosal surfaces. Some antimicrobial factors such as β-defensins or lactoperoxidase even cause direct harm to the pathogens (Caswell & Williams, 2015).

If the particles reach the alveoli, alveolar macrophages are there to recognize foreign microbes. They can phagocytose and kill most microbes without inducing a generalized inflammatory response. The alveolar macrophages are then mainly cleared through the bronchioles by the ciliary movement, even if this system isn't as efficient as further up the bronchioles. Interstitial macrophages phagocytose the particles that penetrate to the pulmonary interstitium.

If the antigens reach the pulmonary lymph nodes and get presented to lymphocytes, a pulmonary immune response is initiated. The inflammatory response may be essential to clear bacteria from the lung but also risk damaging the pulmonary tissue and lung function. (Caswell & Williams, 2015)

Gross pathology

Jensen *et al.* (2008) stated that the localization and appearance of the lung lesions can give a fairly certain indication of pathogenesis and aetiology. The authors divided the concept of pneumonia into bronchopneumonia, interstitial pneumonia and focal pneumonia. Furthermore, they divided bronchopneumonia into lobular or lobar depending on the distribution of the lesions.

Bronchopneumonia

Bronchopneumonia typically have an airborne aetiology. The lesion originates at the bronchiolar-alveolar junction. Then it spreads distally to the alveoli and proximally to the bronchioles. Exudation in the bronchioles is a characteristic part of the pathogenesis (Jensen *et al.* 2008).

Jensen *et al.*, (2008) mentioned that the exudation in the bronchioalveolar system could make it more macroscopically visible and called it the "cloverleaf mark". They described it as several, greyish, up to one millimeter in diameter, circular lesions that is characteristic for bronchopneumonia or an inflammation even more peripherally located. This structure can occur in both the acute and chronic state of bronchopneumonia but is not always present.

The distribution of bronchopneumonia is characteristically cranioventral. It may be explained by increased deposition of inhaled particles in this area because of gravitational influences. There is also evidence that intravenous injection of *Mannheimia haemolytica* can cause pneumonia in the same regions, which could indicate that the lung defenses could be less effective in this area (Caswell & Williams, 2015).

The causes of bronchopneumonia are often opportunistic pathogens that could be found normally in the upper respiratory tract. An afflicted animal can be suffering of increased exposure of bacteria, impaired lung defenses or both. For example, stress can lead to increased numbers of *Mannheimia haemolytica* in the upper respiratory tract which leads to increased numbers of bacteria in the inhaled droplets. Impaired lung defenses could be due to stress, viral and mycoplasmal infection, toxic gases etc. (Caswell & Williams, 2015).

The typical lesions of bronchopneumonia are consolidated with a red-purple, maroon or pink-grey color depending on the age. A color change without the change of texture is often due to hemorrhage or congestion rather than bronchopneumonia. In subacute cases, catarrhal or purulent material could be found in the bronchioles and the cut surface is edematous. In chronic cases the cut surface could be dryer, discolored without exudation and cuts crisply. Bronchopneumonia with a lobular distribution involves some lobules entirely whereas nearby lobules are unaffected. This is mostly visible at the border of the lesion. Bronchopneumonia with a lobar distribution involves an entire consolidated lobe and could be accompanied by pleuritis (Caswell & Williams, 2015).

Interstitial pneumonia

The term "interstitial pneumonia" can be used in different ways in veterinary medicine, but generally it describes inflammation of the alveolar or interlobular septa (Caswell & Williams, 2015).

The typical lesions of interstitial pneumonia are diffuse changes of the consistency with a caudodorsal distribution. The lungs are heavy, firmer and are sometimes bigger than normal, which may cause impressions from the ribs. The lesions are typically bilateral. One example of interstitial lung disease in sheep is pneumonia caused by the maedi-visna virus (Jensen *et al.,* 2008).

The most commonly identified form of interstitial lung disease is diffuse alveolar damage. That involves injury to type I pneumocytes or endothelial cells in the alveolar septa, and results in pulmonary edema, formation of hyaline membranes, proliferation of type II pneumocytes and interstitial fibrosis. There may be some exudation in the airways in the acute phase, but not as in a bronchopneumonia. The subacute phase is characterized by cellular proliferation and the chronic phase by fibrosis (Caswell & Williams, 2015).

The term "bronchointerstitial pneumonia" can be used to describe the presence of both bronchiolar necrosis and diffuse alveolar damage, but it can also be used to describe diseases in which leukocytes infiltrate alveolar septa. Unlike bronchopneumonia, where bronchioles and alveoli are filled with leukocytes as a result of bacterial infection of the airspace and necrosis of the bronchiolar or alveolar epithelium is not usually present (Caswell & Williams, 2015).

Therefore, bronchointerstitial pneumonia is more of a histological diagnosis, and macroscopically it can look like a bronchopneumonia. But unlike bronchopneumonia, the exudation to the airways ends quickly and it starts to look more like an interstitial pneumonia with proliferation of type II pneumocytes and interstitial infiltration dominated by lymphocytes (Jensen *et al.,* 2008).

Focal pneumonia

The typical lesions of focal pneumonia are small, spherical and separate. Small changes with a lobular look can also be called focal, even if they aren't spherical. They are unifocal, bifocal or multifocal. The lesions often have a firm center, caused by necrosis or cell infiltration, that is grey or yellow. The pathogenesis of a focal pneumonia can vary. If the changes are disseminated, they can be of an embolic origin, but they can also show to be airborne and therefore be classified as bronchopneumonia. Mostly, the focal pneumonias are caused by bacteria or parasites. Different species of lungworms causes typical focal pneumonia in sheep (Jensen *et al.,* 2008).

MATERIAL AND METHODS

Sample collection

44 sets of sheep lungs with grossly visible pneumonic lesions were collected from two slaughterhouses located in Uppland in Sweden. The animals were submitted for routine slaughter and passed the ante-mortem inspection done by a veterinarian. Therefore, it was assumed that the animals showed few or none clinical signs of disease. No other details of the sex, breed or husbandry conditions were available.

Following gross post-mortem inspection, all sets of lungs with grossly visible pneumonic lesions were collected during the period of September-October. The investigations of the lungs were performed within two days from when the sheep were slaughtered. They were kept refrigerated until the moment of investigation.

Pathological investigation

Gross pathology

The pulmonary lesions were photographed and then described objectively using the following attributes; distribution, demarcation, contour, shape, color, size, texture, consistency and extent. A schematic picture, where the affected areas got marked with red color, was used to estimate the extent of the consolidation. (Fig. 2) Gimp (http://www.gimp.org), a graphics editor was used to calculate the area of the red area. That gave a more objective estimation even if no regard was taken to the volume of the lungs when the area was calculated. The pneumonia was then scored as mild, moderate or severe depending on the extent of the cranioventral consolidation together with how severe the inflammation was estimated to be in the histopathological investigation.

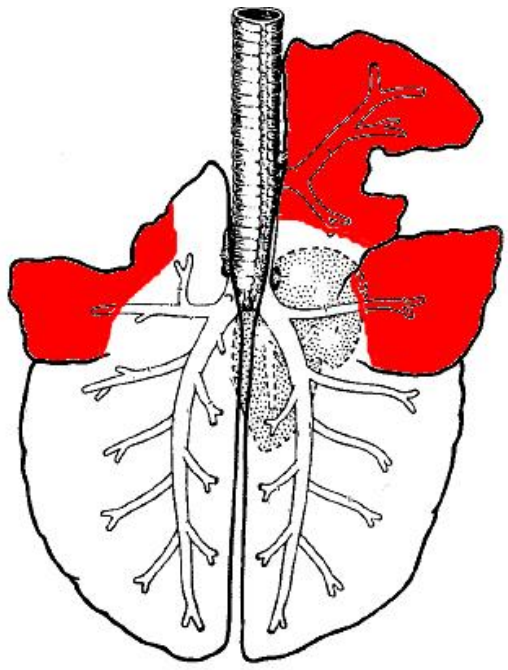

Fig. 2. *Schematic picture over lung number 32, estimated to have 27% consolidation.*

A subjective interpretation of the gross observations was made, based on Jensen *et al.,* (2008) and Caswell & Williams (2015).

Representative samples of affected lung tissue were fixed in 10% buffered formalin.

Histopathology

The histology slides were stained using a standard H&E (haematoxylin and eosin) stain. The following attributes were studied: The extent of the consolidation, the presence of pleuritis, the occurrence of a hyaline scar or a type II pneumocyte proliferation. Following were graded subjectively: The hyperplasia of the bronchiolar epithelia, hyperplasia of the lymphoid tissue and the amount of exudate including neutrophils in the lumen of the airways. If fibrosis were suspected the slides were stained using Masson´s trichrome stain, where connective tissue gets blue. The histopathological investigations were blinded so that no information about the results of the microbiological investigation were available at the time.

Finally, the results were interpreted to conclude if the inflammation were chronic, chronic active or acute and was diagnosed to be a bronchopneumonia, bronchointerstitial pneumonia or an interstitial pneumonia.

Microbiological investigation

Aerobic bacterial culture

An incision through the surface of the lung were performed at the affected area, usually the right cranial lobe, using a sterile scalpel. Then a Liquid-based Collection and Transport system (E-Swab, COPAN Diagnostics) were used to collect the sample from the lung parenchyma at the cut surface. The liquid amies media allows multiple tests to be analyzed from the same specimen.

The analyses were performed at SVA (the Swedish National Veterinary Institute) using an accredited method with relevant growth media. The analyses started within a day from the moment the samples were collected.

The growth media used were horse blood agar, purple lactose agar and horse blood agar incubated in 5% carbon dioxide. Those three petri dishes were then incubated in 37 degrees Celsius overnight and interpreted after 48 hours. Suspected colonies were identified using MALDI-TOF MS (Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry), with focus on colonies looking like *Pasteurella* spp., *Mannheimia* spp. or *Bibersteinia* spp. SVA used the MALDI Biotyper from Bruker. The MALDI Biotyper measures highly abundant proteins and the characteristic patterns of these highly abundant proteins are then used to identify a particular microorganism by matching the respective pattern with an open database. In this case, the database version 6903 were used, with an addition of six strains of *Mannheimia* spp. and 24 strains of *Pasteurella* spp.

Antimicrobial Susceptibility Test

The antimicrobial susceptibility test was performed at SVA.

A microdilution test was used. The test called VetMIC is a MIC-based (Minimum Inhibitory Concentration) system where bacterial material from a Müller Hinton broth is suspended and inoculated into micro titer plates. After incubation the lowest MIC is read as the lowest concentration inhibiting visible growth.

Table 1. *Antibiotics and their tested concentrations.*

Antibiotic	**Concentration**
Penicillin	0,06-0,5 mg/l
Ampicillin	0,5-4 mg/l
Trimethoprim/sulfamethoxazole	0,25/4,75-2/38 mg/l
Enrofloxacin	0,06-0,5 mg/l
Tetracycline	0,5-4 mg/l
Florfenicol	1-8 mg/l

The results were interpreted with the same breakpoints for resistance that were used by SVA (the Swedish National Veterinary Institute) in the report of resistance monitoring, the Swedres-Svarm. (Swedres-Svarm, 2014)

Table 2. *Antibiotic´s breakpoints for resistance*

Penicillin	>0,5
Ampicillin	>1
Trimethoprim/sulfamethoxazole	>4
Enrofloxacin	>0,25
Tetracycline	>2
Florfenicol	>4

Mycoplasma PCR

The PCR (Polymerase Chain Reaction) tests were performed by APHA, Dep. of Bacteriology, Mycoplasma Group, Weybridge.

Statistical significance test

The test used to analyze the results was Fischer´s Exact Test in Minitab 17 (https://www.minitab.com), and the result was interpreted as significant at a p-value <0,05.

RESULTS

Pathological investigation

Gross pathological investigation

44 sets of sheep lungs were collected and examined. Number 2, 6 and 8 were then excluded from the project due to a deviant gross or histological appearance, for example only exhibiting focal abscesses. That leaved 41 sets of sheep lungs that all exhibited the gross appearance of bronchopneumonia with a varying chronicity. None of the lungs collected exhibited the appearance of an interstitial pneumonia.

Fig. 3. *Set of lungs number 1 with sharply demarcated, grayish red colored consolidation affecting all the cranioventral lobes.*

The findings were well demarcated consolidated areas of lung tissue with a cranioventral distribution affecting one or several lobes or lobules (fig. 3). Affected areas were sometimes raised or depressed when compared to unaffected tissue. The lesions were red-brown to purple-gray and had a firm, meaty consistency. The texture was solid and the look of the cut surfaces varied from dark red and edematous to grayish and firm (fig. 5), sometimes with pale grey areas or like in four cases; focal abscesses containing an amorphous yellowish material (fig. 6). The lungs in which at least the right cranial lobe exhibited relatively homogenous atelectasis, discoloration and a meaty consistency, were classified as "Type 1" which included 35 sets of lungs. The lungs that didn't fit the description were classified as "Type 2" which included 6 sets of lungs. Fig. 4 shows examples of both types.

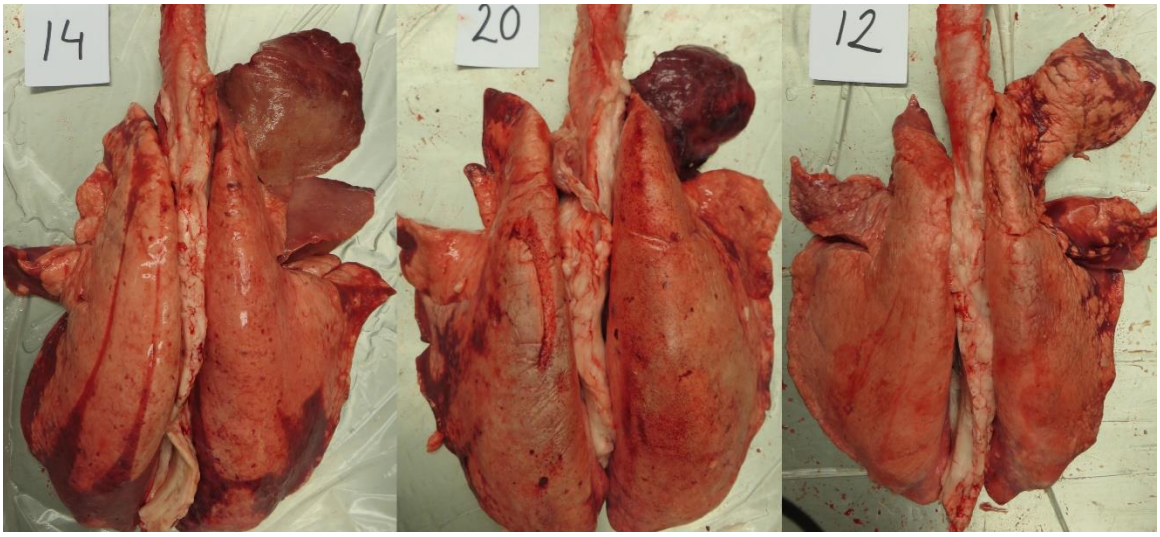

Fig. 4. *Lungs number 14 and 20 fit the description and is therefore of Type 1. Lungs number 12 is of Type 2.*

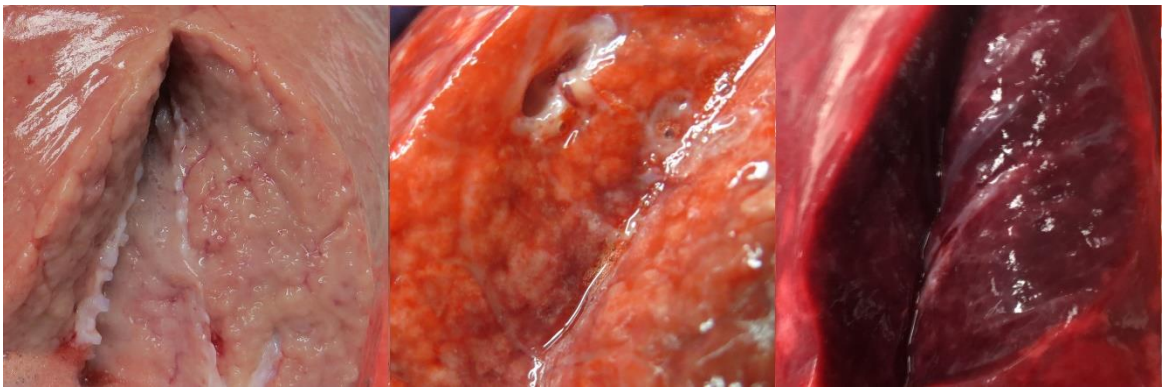

Fig. 5. *The variating look of cut surfaces at lungs number 14, 15 and 28 (from the left). Number 14 also showing mucous exudate in the bronchus.*

Fig. 6. *Number 33 with the largest abscess that had a diameter of 5 cm. Other abscesses rarely exceeded 1 cm in diameter.*

25 of the lungs (61%) had clearly visible mucous to mucopurulent exudate in the airways (fig. 5).

Evidence of pleuritis was grossly visible in 19 (46%) of the animals. This consisted of a matte finish of the visceral pleura or adherences between lobes and/or other surfaces (fig. 7).

Fig. 7. *Pleuritis and petechiae in lungs number 11*.

29 of the lungs (71%) showed subpleural bleedings, like petechiae or echymosis (Fig. 7). The bleedings were often seen adjacent to the lesions and were accompanied by hyperemic lymph nodes.

Three of the lungs were estimated to have an affected area of <3% in the schematic picture, 31 of the lungs were estimated to have an affected area of 10-20% and 7 lungs was estimated to have an affected area of >20%.

Histopathological investigation

On histopathological investigation, all the slides were dominated by severe consolidation which made the interpretation of the interstitium difficult (Fig. 10).

The following lesions were observed: pleuritis, intra-alveolar or intrabronchiolar exudation dominated by neutrophils (Fig. 8, Fig. 11), bronchiolar epithelial hyperplasia, peribronchial/peribronchiolar lymphoid hyperplasia (Fig. 9) and hyaline scarring (Fig. 12, Fig. 13). A widening of the alveolar septa was often noted in the interstitium (Fig. 10), either due to infiltration of leukocytes, fibrosis or edema. Proliferation of type II pneumocytes were seen in a few cases, but that finding can be unrecorded in several cases due to the severe atelectasis that made it hard to recognize the lesion with certainty. Other less frequently observed lesions included emphysema/air trapping, a widening of interlobular septa due to edema or fibrosis, bronchiectasis or bronchiolar proliferation.

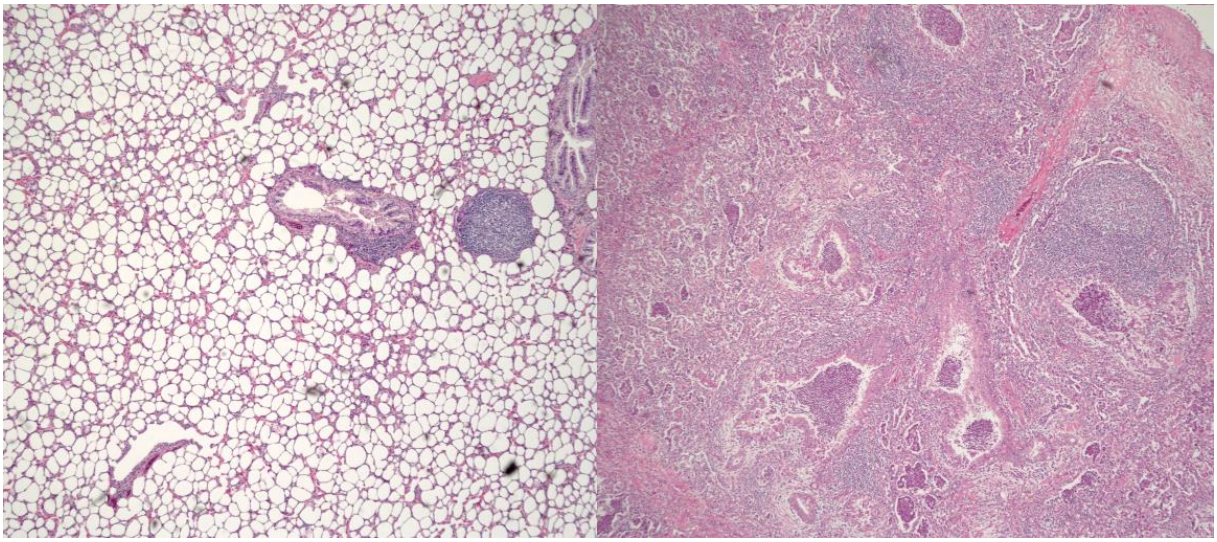

Fig. 8. *Normal tissue (left) versus an overview of the tissue in lungs number 42 (right). Haematoxylin and eosin stain, 4X.*

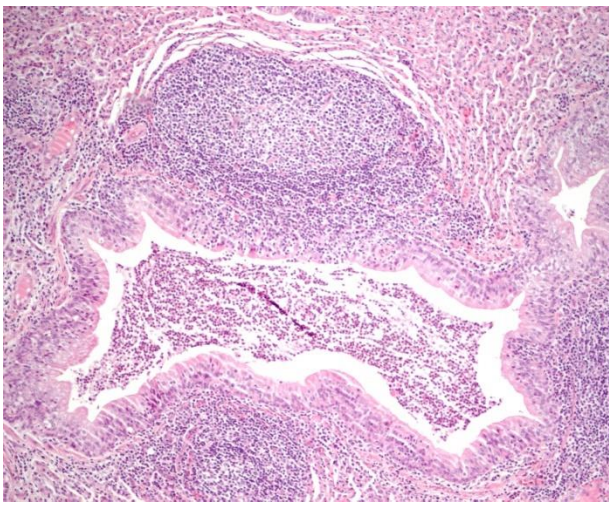

Fig. 9. *Bronchiolar epithelial hyperplasia, peribronchiolar lymphoid hyperplasia and intrabronchiolar exudation. Haematoxylin and eosin stain, 10X, lungs number 1.*

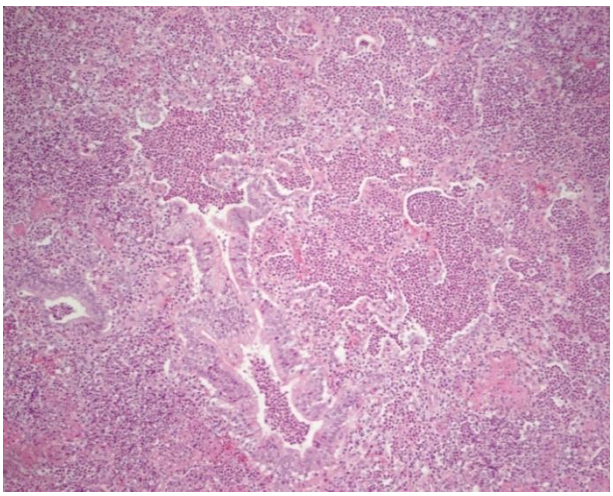

Fig. 11. *A severe amount of intra-alveolar and intrabronchiolar exudation in lungs number 16. Haematoxylin and eosin stain, 10X.*

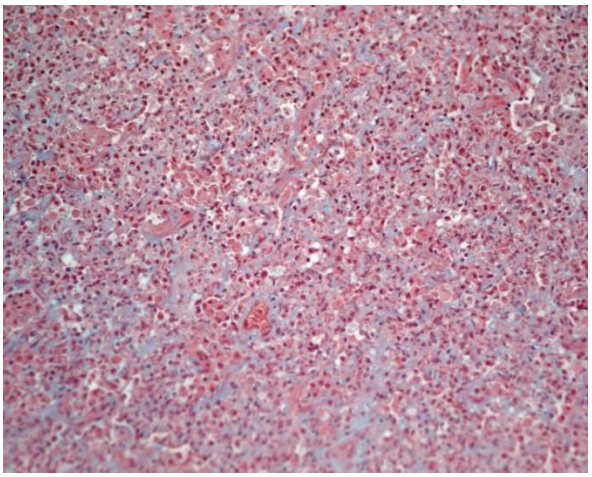

Fig. 10. *Widened alveolar septa, atelectasis and fibrosis (blue) in lungs number 17. Masson´s trichrome stain, 20X.*

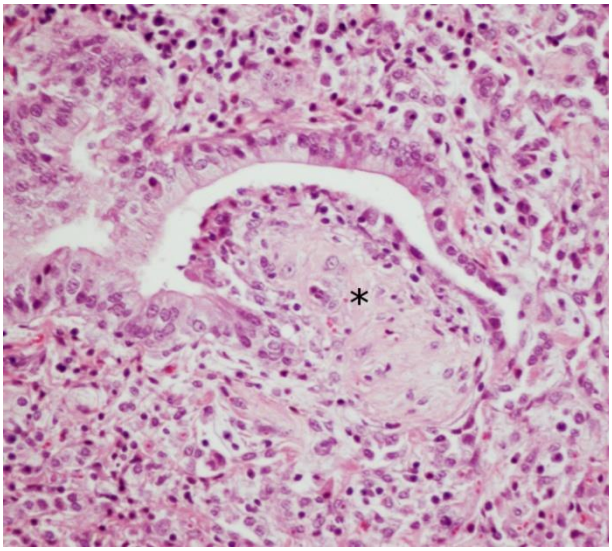

Fig. 12. *Hyaline scar (asterisk), causing a stenosis of the bronchiolar lumen in lungs number 1. Haematoxylin and eosin stain, 20X.*

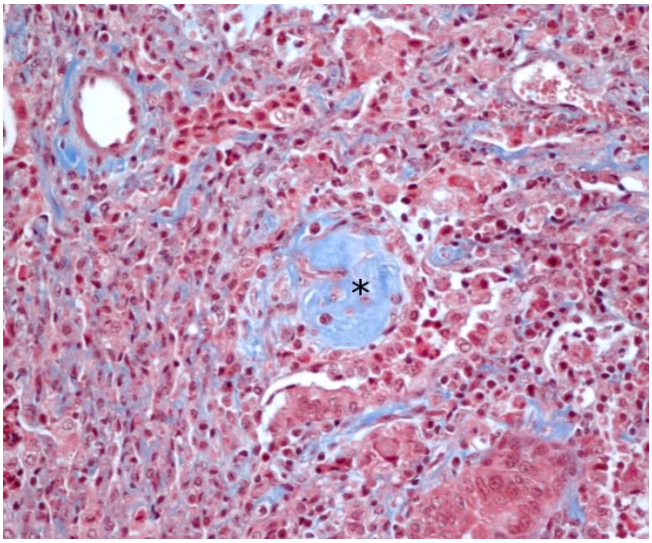

Fig. 13. *Hyaline scar (asterisk) in a Masson's trichrome stain, lungs number 4, 20X.*

28 of the lungs (68%) had signs of pleuritis at the histopathological examination. In the majority of cases it was a mild, chronic pleuritis that wasn't visible in the gross examination. The most common findings were bronchiolar epithelial hyperplasia (98%), peribronchial/peribronchiolar lymphoid hyperplasia (98%), intrabronchiolar/intraalveolar exudate (95%) and a widening of the intra-alveolar septa (88%). Hyaline scarring was observed in 14 lungs (34%). The scars were most easily observed in the Masson´s trichrome stain where they are seen as blue (Fig. 13). In lungs number 44, lungworms were found in a part of the tissue. They were interpreted as an incidental finding and didn't seem to be connected to the pneumonia. All the lungs had atelectasis. None of the lungs had signs of ovine pulmonary adenocarcinoma (OPA or Jaagsiekte), a disease not considered present in Sweden.

These inflammatory changes were together with the estimated area of the lesions then used to score the pneumonia as mild, moderate or severe. In eight animals, the pneumonia was graded as mild, in 26 of the animals it was graded as moderate and in the remaining seven this parameter was designated severe.

The lung biopsies with inflammation classified as acute/subacute were associated with bronchopneumonias in contrast to the lesions that were classified as chronic which were associated with bronchointerstitial pneumonias, with a statistical significance and a p-value of 0,018182.

Microbiological investigation

Mycoplasma ovipneumoniae were detected via PCR in 31 lungs which is 76% of the total of 41 lungs. In 5 of them *Mycoplasma ovipneumoniae* were accompanied by *Mycoplasma arginini. Mycoplasma arginini* was also detected alone in additional three lungs, therefore *Mycoplasma arginini* was present in almost 20% of the lungs.

The most common bacteria isolated in the aerobic bacterial culture was *Mannheimia haemolytica.* It was isolated in 26 lungs (63%), and always together with a *Mycoplasma* spp. In lungs number 4, *Streptococcus dysgalactiae* subsp. *equisimilis* was isolated (earlier name *Streptococcus equisimilis*). Lungs number 33 and 34 had several abscesses with similar looking greenish white exudate (fig. 5). In these cases, two samples were taken, one usual from the cut surface of the ordinary affected parenchyma and one from the exudate in an abscess. In lungs number 33, no specific infectious agent could be isolated from the parenchyma, but *Bibersteinia trehalosi* was isolated from the abscess. In lungs number 34, *Mannheimia haemolytica* was isolated from the parenchyma but no specific agent could be isolated from the mixed bacterial culture of the abscess.

Table 3. *Results of the pathological and microbiological investigations*

	Gross pathology						Histopathology									Microorganisms		
No.	S	T	Pl	GEx	SpB	Ab	HPl	EH	LH	HEx	HS	WA	C	In	Comment	Culture	PcS	Myc PCR
1	sev	1	-	+	-	-	-	3	3	3	+	2	CA	BI	Wide interlobular septa	*M.h*	S	*M.o*
2		2													Excluded			
3	sev	1	+	+	p	+	2	3	3	3	+	3	CA	BI		-		-
4	mld	2	-	+	-	-	-	2	3	2	+	1	CA	BP		*S.e*	S	*M.o, M.a*
5	mod	1	-	+	p	-	-	3	2	3	-	2	CA	BI		*M.h*	S	*M.o*
6		2													Excluded			
7	mod	1	-	-	-	-	-	2	3	2	-	2	CA	BP	Air trapping	-		-
8		2													Excluded			
9	mld	2	-	-	-	+	-	-	1	-	-	2	C	BI		-		-
10	mod	1	-	-	-	-	1	1	2	3	-	1	A	BI		*M.h*	S	*M.o*
11	mod	1	+	+	p, e	-	3	2	2	2	+	2	CA	BI		*M.h*	S	*M.o*
12	mld	2	+	-	-	-	1	3	3	2	-	1	CA	BP		-		-
13	mod	1	+	+	p, e	-	1	3	3	2	+	2	CA	BI		*M.h*	S	*M.o*
14	sev	1	-	+	-	-	1	3	3	3	-	1	CA	BP		-		*M.o*
15	mod	1	-	-	p, e	-	3	2	1	3	-	1	A	BP		*M.h*	S	*M.o*
16	mld	1	+	+	e	-	2	2	-	3	-	1	A	BP	Wide interlobular septa	-		*M.o*
17	mld	1	+	-	e	-	2	2	2	1	-	2	CA	BI		-		*M.o*
18	mod	1	+	+	-	-	1	2	2	3	-	2	CA	BI		*M.h*	S	*M.o*
19	mld	2	+	-	p, e	-	1	2	1	-	-	3	C	BI	Bronkiektasi. Majority of lymphocytes.	-		-
20	mod	1	+	-	p	-	1	2	1	2	-	1	A	BP		*M.h*	S	*M.o*
21	mod	1	-	+	p	-	1	2	3	2	-	2	CA	BI		*M.h*	S	*M.o*
22	mod	1	+	+	p	+	1	3	3	2	-	2	CA	BI	Wide interlobular septa	-		*M.o*
23	mld	2	-	-	-	-	1	3	3	2	-	1	CA	BP	Air trapping. Bronkiektasi.	-		-
24	mod	1	+	+	e	-	2	3	3	1	-	2	CA	BI		-		-
25	mod	1	-	+	p	-	-	2	3	2	+	2	CA	BI		*M.h*	S	*M.o, M.a*
26	mod	1	-	+	-	-	1	3	3	3	-	2	CA	BI		*M.h*	S	*M.o, M.a*
27	mod	1	-	-	p	-	-	3	3	1	+	1	CA	BP		-		*M.o*
28	sev	1	-	-	p, e	-	-	1	1	3	-	1	A	BP		*M.h*	S	*M.o*
29	mld	2	-	+	p	-	1	2	1	2	-	-	CA	BP		*M.h*	S	*M.o*
30	mod	1	+	+	p, e	-	-	2	2	2	-	-	CA	BP		*M.h*	S	*M.o, M.a*
31	mod	1	-	+	e	-	-	2	2	2	-	-	A	BP	Severe bleedings	-		*M.o*
32	sev	1	+	-	-	-	-	3	2	2	+	2	CA	BP		*M.h*	S	*M.o*
33	mod	1	+	+	p, e	+	2	3	2	2	+	2	CA	BI	Wide interlobular septa. Fibrosis. Majority of lymphocytes.	*B.t*	S	*M.a*
34	mod	1	+	+	p	+	2	3	3	2	+	2	C	BI	Wide interlobular septa. Fibrosis.	*M.h*	S	*M.a*
35	mod	1	-	+	p	-	1	2	1	2	+	-	CA	BP		*M.h*	S	*M.o*
36	mod	1	-	-	p	-	1	1	1	3	-	1	A	BP	Air trapping	*M.h*	S	*M.o*
37	mod	1	+	-	p, e	-	1	2	3	2	-	-	CA	BP	Air trapping.	*M.h*	S	*M.a*
38	mod	1	-	+	p	-	-	3	2	3	+	1	CA	BP		*M.h*	S	*M.o*
39	mod	1	-	+	p, e	-	-	2	2	3	-	1	A	BI	Air trapping	*M.h*	S	*M.o*
40	mod	1	-	+	p	-	1	3	3	3	+	1	CA	BI	Bronkiektasi or proliferation	*M.h*	S	*M.o, M.a*
41	mod	1	-	-	-	-	1	2	2	3	-	1	CA	BP	Wide interlobular septa with edema.	*M.h*	S	*M.o*
42	mod	1	+	+	p, e	-	1	3	3	3	+	2	CA	BI	Mild fibrosis.	*M.h*	S	*M.o*
43	sev	1	+	-	p, e	-	3	2	2	3	-	1	CA	BP		*M.h*	S	*M.o*
44	sev	1	+	+	p, e	-	3	2	3	3	-	1	A	BP	Parasites as an incidental finding	*M.h*	S	*M.o*

Key to table: +, present; -, absent; 1, mild; 2, moderate; 3, severe; **S**, severity of lesions; mld, mild; mod, moderate; sev, severe; **T**, type of appearence; 1, Type 1; 2, Type 2; **Pl**, evidence of pleuritis at gross examination; **GEx**, signs of exudate in airways at gross examination; **SpB**, visible subpleural bleeding (p, petechiae; e, echymosis); **Ab**, abscessformations; **HPl**, visible pleuritis in histopathological examination; **EH**, bronchiolar epithelial hyperplasia; **LH**, peribronchial/peribronchiolar lymphoid hyperplasia; **HEx**, intrabronchiolar/intra-alveolar exudate; **HS**, hyaline scarring; **WA**, widening of alveolar septa due to cells, fibrosis or edema; **C**, estimated chronicity (A, acute or subacute; CA, chronic active; C, chronic); **In**, subjective interpretation of histopathological findings (BP, bronchopneumonia; BI, bronchointerstitial pneumonia); **PcS**, penicillin sensitivity of isolated bacteria (S, sensitive); **Myc PCR**, Mycoplasma PCR; *M.h, Mannheimia haemolytica* detected; *S.e, Streptococcus equisimilis* detected; *B.t, Bibersteinia trehalosi* detected; *M.o, Mycoplasma ovipneumoniae* detected; *M.a, Mycoplasma arginini* detected.

Mannheimia haemolytica is associated with pneumonias graded as moderate or severe with a statistical significance and a p-value of 0,0018.

All isolated bacteria in the aerobic bacterial culture were found sensitive to penicillin in the antimicrobial susceptibility test.

Mycoplasma ovipneumoniae is associated with the features of the lesions described in this project as "Type 1" with statistical significance. The result was significant both if the former excluded lungs 2,6 and 8 that were "Type 2" and negative for *Mycoplasma* spp., were included, or if only the other 41 lungs that were part of the whole project were included in the calculation. The p-value were between 0,012-0,00005 depending on who made the grouping into the two types, since some lungs that is borderline cases can be interpreted different. Either way, the result was statistically significant with a p-value <0,05.

No specific histopathological findings could be correlated to *Mycoplasma ovipneumoniae* or *Mycoplasma* spp.

The results of the pathological and microbiological investigation are presented in table 3.

DISCUSSION

The aim of this project was to examine the prevalence of *Mycoplasma ovipneumoniae* amongst sheep with pneumonic lesions found at routine slaughter and to see if a specific infectious agent could be correlated to a specific gross or histological appearance.

In this study, *Mycoplasma ovipneumoniae* was found in 76% of the lungs and *Mannheimia haemolytica* was found in 63% of the lungs, always in combination with a *Mycoplasma* spp. That reminds of the result of Sheehan *et al.* (2007) who collected 30 sheep lungs with grossly visible signs of chronic bronchopneumonia. They used bacterial and virus isolation, fluorescent antibody tests and immunohistochemistry to identify potential causative agents such as *Mycoplasma ovipneumoniae, Mannheimia haemolytica,* parainfluenza type 3-virus and respiratory syncytial virus. They found *Mycoplasma ovipneumoniae* in 27 of the 30 lungs (90%) and *Mannheimia haemolytica* in 9 lungs, even then always in combination with *Mycoplasma ovipneumoniae.*

These results suggest that *Mycoplasma ovipneumoniae* have an non negligible role as a causative agent of bronchopneumonia in sheep. Instead, it seems to be an important part of the aetiology, especially since the more fatal *Mannheimia haemolytica* does not seem to occur by its own. Whether *Mycoplasma ovipneumoniae* can cause fatal pneumoniae without involvement of another infection remains unanswered. It was identified by its own in six of the lungs. Two of them were classified as mild pneumonia, three as moderate and one of them as severe. In this project the lungs were not tested for viruses, but Sheehan *et al.* (2007) tested for viruses and they also found several lungs where *Mycoplasma ovipneumoniae* seemed to act alone as a causative factor. In the cases where no causative agent could be isolated, viruses may be the reason as they also can cause bronchopneumonia (Caswell & Williams, 2015).

These sheep all passed the ante-mortem inspection and therefore it is assumed they did not show any signs of disease. They were examined not only by a veterinarian but probably also by the owner itself, who is responsible of not sending ill animals to normal slaughter. Mycoplasmosis is often described as subclinical or low pathogenic, which may explain why it is found in such a high prevalence at routine slaughter. But *Mannheimia haemolytica* was also found in a high prevalence, which is more surprising since it´s associated with a more acute and severe disease. The lesions of the lungs were also classified as moderate or severe in high proportions, even if it would be more reasonable to expect a higher proportion of mild lesions at routine slaughter and few to no severe lesions. It may be an evidence of the unwillingness of sheep to show signs of disease, due to the reason that it makes them vulnerable to predators. And that fact can also suggest that they are suffering more of mycoplasmosis than we have acknowledged before because of the sheep ability to mask signs of clinical disease.

To conclude, *Mycoplasma ovipneumoniae* seems to in general be part of a low pathogenic disease with high morbidity and low mortality (Radostits *et al.,* 2007; Ayling & Nicholas, 2007) but have a negative impact on the lungs defenses (Al-Kaissi & Alley, 1983; Niang *et al.,* 1998) and is therefore predisposing to secondary invaders that can cause a more serious disease (Alley *et al.,* 1999). *Mycoplasma ovipneumoniae* may cause a more serious disease on its own if there are enough adverse environment conditions in combination with a high prevalence of *Mycoplasma ovipneumoniae* (Mohkber Dezfouli *et al.,* 2011; McAuliffe *et al.,* 2003), but there is a need for more studies on that part.

Mycoplasma arginini did also occur amongst the results of the PCR investigation. Either in combination with *Mycoplasma ovipneumoniae* and *Mannheimia haemolytica,* together with only *Mannheimia haemolytica* or even together with *Bibersteinia trehalosi* in one case. It did never occur by its own. The pathogenicity of *Mycoplasma arginini* is quite unknown, especially since it appears in a lower frequency than *Mycoplasma ovipneumoniae* in most studies which could be a sign for a lack of a role for the organism (Lin *et al.,* 2008). On the other hand, it was once found in a higher frequency in a survey made in Iran (Tabatabayi *et al.,* 1992).

In this project, *Mycoplasma ovipneumoniae* is associated with the features of the lesions described in this project as “Type 1” with a strong statistical significance, suggesting that it may be possible to roughly diagnose the pneumonia by the gross appearance. With that in mind, there are always lungs that are exceptions from the rule. About 14% of the lungs characterized as “Type 1” in this project didn´t have *Mycoplasma ovipneumoniae.* Microbiological investigation is necessary to confirm the diagnose.

The slides that were interpreted as bronchointerstitial pneumonias did often have chronic changes of the bronchioles which suggests that they´ve all started as bronchopneumonias and that it is a matter of chronicity if the changes of the interstitium reaches the level until it gets interpreted as a bronchointerstitial pneumonia. That hypothesis is supported by the statistically significant association of bronchopneumonias to acute or subacute inflammations and of bronchointerstitial pneumonias to chronic inflammations. Whether in the group of chronic active inflammations, both bronchopneumonias and bronchointerstitial pneumonia occurred in almost the same level. That suggests that the histological classification into bronchopneumonias or bronchointerstitial pneumonias tells more about the chronicity than the aetiology in this setting.

All the isolates of *Mannheimia haemolytica* were sensitive to penicillin in the antimicrobial susceptibility test. Therefore, there´s no reason to change the recommendation that penicillin is the first choice of treatment in a pasteurellosis pneumonia. On the other hand, both in this project and in the study by Sheehan *et al.* (2007), *Mannheimia haemolytica* did never occur in the absence of *Mycoplasma ovipneumoniae* which has a natural resistance to penicillin. A combined vaccine against both pasteurellosis and mycoplasmosis would be the best solution and would reduce the use of antibiotics. In the specific case of a flock with a coughing problem, transtracheal bronchoalveolar lavage may be a reasonable diagnostic solution since the detection of a causative agent in the lavage fluid is closely correlated to clinical disease, in the contrast to nasal swabs (Sheehan *et al.*, 2005).

A suggestion for a further study is a field study were coughing groups of sheep and a control group is tested by bronchoalveolar lavage. Sheep that show clinical signs may be experimentally treated depending on the results of the bronchoalveolar lavage to examine the success rate of the treatment.

It would also be interesting to know what would happen to the sheep with *Mannheimia haemolytica* if they hadn´t gone to slaughter. Would they have gotten worse quickly, or can *Mannheimia haemolytica* also be part of a pneumonia with low mortality?

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