ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Mycoplasmosis: An Emerging Threat to Developing Livestock Industry

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Manuscript Info

Manuscript History:

Received: 12 November 2013
Final Accepted: 24 December 2013
Published Online: January 2014

Abstract

Introduction

The *Mycoplasma* is the smallest free-living and pleomorphic organism ranging from 0.3-1.0µm in diameter. It can be passed through bacteria-retaining filters of pore size 0.22 to 0.45µm (Quin et al., 2001). In contrast to bacteria, *Mycoplasmas* have no cell wall but are bounded by a plasma membrane. *Mycoplasma*s lack cell walls and are, therefore, a) pleomorphic and b) resistant to antibiotics of the betalactamine group, such as penicillin. Growth of *Mycoplasma* is relatively fastidious and requires special media rich in cholesterol. It is important to note that the lack of a cell wall allows the organism to be plastic, which means that its morphology is questionable and exists in several ways. These cell shapes presumably contribute to the ability of *Mycoplasma*s to thrive in their respective environments. Different species of *Mycoplasma* in different media might be found as filamentous, coccoidal, spherical, or granular. The cells often present a certain shape, with a characteristic small size. Most are pseudococcoidal. It is the filamentous and branching forms that the name *Mycoplasma* came from, meaning "fungus form." This plasticity serves many purposes, including aiding in the physical stability of the organism. However, this same feature can make them a difficult organism to study and in the past, there were several erroneous estimates to even the size of *Mycoplasma*s because of their flexibility, which allowed them to pass through filters with pore sizes smaller than the true diameter of the cell. Their ability to survive is also aided by the osmotically protected habitat of an animal's body. Also making them unique from other bacteria is their genome size, which is up to 1/5 the size of *E.coli*. Ranging between 800-1200 kb pairs, their molecular weight and chromosomes are smaller than that of any free-living prokaryote. It is not surprising that some of the sequences missing are those that code for proteins related to cell wall functions. The G-C content of *Mycoplasma* is another interesting feature at 23 – 40% of the DNA. They are capable of a gliding motility, where the cell attaches to various matrices and slides on them (Quin et al., 2001). This ability to adhere and move is critical for *Mycoplasma* so it can move to ideal locations within the host and colonize various tissues. This is one of the things that make them such an effective and specific pathogen.

They can be pathogenic to humans, animals and plants and really are quite unique in many ways. Their structure is uncommon in nature due to the lack of a cell wall. Due to the lack of a cell wall, they are a trickier organism to relate to the infection.

*Mycoplasma*s are unusual among bacteria in that most require sterols for the stability of their cytoplasmic membrane (Maniloff, J. and Morowitz, H.J., 1972). Sterols are acquired from the environment, usually as cholesterol from the animal host. Division occurs by budding, with the cells remaining attached or connected by thin hyphae-like

material. The method of gene transfer is likely cell fusion or conjugation. As their structures are dynamic, so is their pattern of growth. It is highly dependent on the media it is growing in. *Mycoplasma* cultures on agar have this characteristic "fried egg" appearance. The ideal pH for growth is 7.6 – 8.0, but it can vary depending on the species. For growth, they also require proteins, amino acids, including purine and pyrimidine, nucleic acids, pentose, lipids, various vitamins and several inorganic materials. The ideal temperature is 37°C and certain osmotic and gaseous conditions must exist. Their morphological heterogeneity, membrane structure, chemical composition, nutritional and physical requirements and metabolic and biosynthetic activities make them a unique and prosperous pathogen. They are not the simple organisms that they were once thought to be and they will increasingly become a subject of study in the years to come. Perhaps, these cellular wonders will give us the ability to unravel once and for all the requirements for independent life. And from there, the possibilities are endless.

Pathogenesis:

The exact mechanism of pathogenesis is still unclear, these factors may involved in pathogenesis.

A. Adherence factors

The mycoplasmas are extracellular pathogens that adhere to epithelial cell surfaces. Thus, adherence proteins are one of the major virulence factors. The adherence protein has been identified as a 168kD protein called P1. The P1 Adhesin localizes at tips of the bacterial cells and binds to sialic acid residues on host epithelial cells. Colonization of the respiratory tract results in the cessation of ciliary movement. The normal clearance mechanisms of the respiratory tract do not function, resulting in contamination of the respiratory tract and the development of a dry cough.

B. Toxic Metabolic Products

The intimate association of the mycoplasma and the host cells provides an environment in which toxic metabolic products accumulate and damage host tissues. Both hydrogen peroxide and superoxide, which are products of mycoplasma metabolism, have been implicated in pathogenesis since oxidized host lipids have been found in infected tissues. Furthermore, the mycoplasmas have been shown to inhibit host cell catalase thereby increasing the peroxide concentrations.

C. Immunopathogenesis

Mycoplasmas can activate macrophages and stimulate cytokine production and lymphocyte activation. Thus, it is has been suggested that host factors also contribute to pathogenesis. Experimental evidence in animals supports this suggestion.

Diseases caused by *Mycoplasma*tales (Jones et al., 1997)

Organism	**Disease**
Bovine *Mycoplasma*s	
M. mycoides subsp mycoides (SC type)	Contagious bovine pleuopneumonia
M. dispar	Respiratory commensal, subclinical or clinical pneumonia
M. alkalescens	Arthritis in calves, isolated from mastitis, genital commensal
M. bovigenitalium	Mastitis, chronic seminal vesiculitis, arthritis in calves, frequent commensal of vagina, seminal vesicles and prepuce, endometritis, vulvovaginitis, suggested as a cause of infertility
M. bovirhinis	Mild mastitis, frequent respiratory commensal, often isolated from pneumonia
M. bovis	Severe mastitis, arthritis, associated with pneumonia, respiratory commensal
M. californicum	Acute mastitis
M. canadense	Mastitis, calf arthritis, respiratory and genital commensal
M. bovoculi	Conjunctivitis, keratocunjunctivitis

M. arginini	Respiratory, conjunctival and genital commensal of cattle, sheep and goats; recovered from dogs
Ovine and Caprine *Mycoplasma*s	
M. mycoides subsp capri and strain F38	Contagious caprine pleuropneumonia
M. mycoides subsp mycoides (LC type)	Pneumonia, arthritis, mastitis, septicemia
M. agalactiae	Contagious agalactia, arthritis, pneumonia, keratoconjunctivitis, vuvovaginitis
M. capricolum	Polyarthritis with septicemia in goats, conjunctivitis, mastitis, vuvlovaginitis, balanoposthitis
M. conjunctivae	Conjunctivitis and keratoconjunctivitis
M. ovipneumoniae	Chronic interstitial pneumonia, mastitis
M. putrefaciens	Mastitis in goat
***Mycoplasma*s of Swine**	
M. floccculare	Mild focal mononuclear pneumonia, respiratory and conjunctival commensal
M. hyopneumoniae	Enzootic pneumonia
M. hyosynoviae	Polyarthritis
Avian *Mycoplasma*s	
M. gallisepticum	Chronic respiratory disease and infectious sinusitis
M. syanoviae	Mild upper respiratory disease and reduced egg production
M. iowae	Reduced hatchability and poor quality poult
Other *Mycoplasma*s	
M. cynos	Pneumonia in dogs
M. canis	Possibly not pathogenic
M. felis	Conjunctivitis in cats

Contagious bovine pleuropneumonia (CBPP)

Contagious bovine pleuropneumonia (CBPP) is an infectious and highly contagious disease of cattle and water buffaloes (Hutyra *et.al.*,1938) considered to be amongst the most important infectious diseases. Affected animals have difficulty in breathing due to damage to the lungs, lose condition and a proportion die. All ages of cattle are susceptible but young cattle develop joint swellings rather than lung infections. Many cattle show no disease signs despite being infected and others recover quickly after a transient mild disease, yet they can carry infection for as long as two years and may be responsible for passing on infection at a later date (Masiga *et.al.,* 1996).

Aetiology

CBPP is caused by *Mycoplasma mycoides* subsp. *mycoides* Small Colony variant (bovine biotype) (*Mmm*SC). This is a member of the 'mycoides cluster,' a grouping of six closely related *Mycoplasma*s that are all pathogenic to a

greater or lesser degree in ruminants. Members of them cluster have a high degree of serological and DNA relatedness (Quin et al., 2001).

Mode of Transmission:

- Transmission occurs mostly by direct contact, droplets emitted by coughing animals, saliva and urine. Transmission up to several kilometers has been suspected under favourable climatic conditions
- Transplacental infection can occur
- Inapparent carriers are a major source of infection
- Cattle movement is important in the spread of the disease

Incubation Period

Incubation period is 1-3 months (sometimes longer)

Clinical Signs

- In adults moderate fever with respiratory, pulmonary and pleuretic symptoms: polypnoea, characteristic attitude (elbows turned out, arched back, head extended), cough dry at first and later becomes moist. When the animal gets up or after exercise, breathing becomes laboured and grunting can be heard
- At percussion, dull sounds can be noticed in the low areas of the thorax
- CBPP often evolves into a chronic disease, characterized by ill thrift and recurrent low grade fever that may be difficult to recognize as pneumonia
- Forced exercise may precipitate coughing
- The course of the disease is usually one to three weeks. Recovered animals retain sequestra in lungs in which the infection remains latent. Stress may cause relapse.
- Pulmonary tropism is not the general rule and infected calves present arthritis with swelling of the joints

Co-existence of pulmonary symptoms in adults and arthritis in young animals should alert the clinician to a diagnosis of CBPP.

Post-Mortem Findings

Important amount of yellow or turbid exudate in the pleural cavity (up to 30 litres) that coagulates to form large fibrinous clots. Fibrinous pleurisy: thickening and inflammation of the pleura with fibrous deposits. Interlobular oedema, marbled appearance due to hepatisation and consolidation at different stages of evolution usually confined to one lung. The presence of unequally distended lymph spaces usually give a beaded appearance to interlobular septa. In long standing cases, zones of necrosis within groups of lobules tend to be sequestrated from the adjacent lung and surrounded by a dense fibrous capsule.

Contagious Caprine Pleuropneumonia (CCPP)

Importance

Contagious caprine pleuropneumonia (CCPP) is one of the most severe diseases of goats. This disease, which affects the respiratory tract, is extremely contagious and frequently fatal; in naive flocks, the morbidity rate may reach 100% and the mortality rate can be as high as 80%. CCPP causes major economic losses in Africa, Asia and the Middle East, where it is endemic. Definitive diagnosis can be difficult. The causative agent is one of the most fastidious *Mycoplasma*s and can be missed during routine bacteriological analysis. It is also closely related to several other species of *Mycoplasma*, which complicates identification and serological screening.

Etiology

Contagious caprine pleuropneumonia is caused by *Mycoplasma capricolum* subsp. *Capripneumoniae*. *M. capripneumoniae* belongs to a closely related group of *Mycoplasma*s called the *Mycoplasma mycoides* cluster. Two other organisms in this group, *M. mycoides* subsp. *capri* and *M. mycoides* subsp. *mycoides* large-colony type, can cause a disease in small ruminants that resembles CCPP but may have extrapulmonary signs and lesions. *M. mycoides mycoides* has also been isolated from goats with pneumonia. This agent (the so-called large colony or LC

variant of *M. mycoides mycoides*) usually produces septicemia, polyarthritis, mastitis, encephalitis, conjunctivitis, hepatitis, or pneumonia in goats.

Incubation Period

The incubation period can be as short as 6 to 10 days but may be very prolonged (3-4 weeks) under natural conditions

Species Affected

Goats are the primary hosts for *M. capripneumoniae* and the only domesticated animals proven to be affected by this organism. However, at least two papers have reported the occurrence of *M. capripneumoniae* in healthy or sick sheep.

Mode of Transmission

Contagious caprine pleuropneumonia is highly contagious. This disease is transmitted during close contact by the inhalation of respiratory droplets. Chronic carriers may exist, but this remains unproven.

Clinical Signs

Contagious caprine pleuropneumonia is strictly a respiratory disease. Peracute, acute and chronic forms may be seen in endemic areas. Peracutely affected goats can die within 1 to 3 days with minimal clinical signs. In acute disease, the initial signs are a very high fever (41-43^{o}C [106-109^{o}F]) lethargy and anorexia, followed within 2 to 3 days by coughing and labored respiration. The cough is frequent, violent and productive. In the final stages of disease, the goat may not be able to move and stands with its front legs wide apart, and its neck stiff and extended. Saliva can drip continuously from the mouth, and the animal may grunt or bleat in pain. Frothy nasal discharge and stringy saliva may be seen terminally. Pregnant goats can abort. Acutely affected goats generally die within seven to 10 days. Chronic CCPP is characterized by a chronic cough, nasal discharge and debilitation. In addition, mild acute infections with fever and cough.

Post-Mortem Findings

The lesions of contagious caprine pleuropneumonia are limited to the respiratory system. Acute disease is characterized by unilateral or bilateral pneumonia and serofibrinous pleuritis with straw-colored fluid in the thorax. On cut surface, the lung is granular with copious straw-colored exudate. Pea-sized, yellow nodules may be found in the lungs; these nodules are surrounded by areas of congestion. Varying degrees of lung consolidation or necrosis can be seen and the regional (bronchial) lymph nodes are enlarged. Some long-term survivors have chronic pleuropneumonia or chronic pleuritis, with encapsulation of acute lesions and numerous adhesions to the chest wall. The interlobular septa is not thickened in domesticated goats.

Morbidity and Mortality

CCPP is severe and highly contagious in naive animals. During outbreaks, goat flocks have morbidity rates up to 100% and mortality rates as high as 80%. The mortality rate can reach 100% in experimentally infected goats.

Differential diagnosis

The differential diagnosis includes pasteurellosis and other forms of bacterial pneumonia, peste des petits ruminants and caseous lymphadenitis. Some other *Mycoplasma*s, particularly *Mycoplasma mycoides* subsp. *capri* and *Mycoplasma mycoides* subsp. *mycoides* large-colony type, can also cause pleuropneumonia resembling CCPP.

Chronic Respiratory Disease (CRD)

The disease has been reported in chickens and turkeys. Mycoplasmosis is an important disease problem in poultry industry, caused by four commonly recognized pathogens – *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, *Mycoplasma meleagridis* and *Mycoplasma iowae* (Bradbury, 2001). It is associated with slow onset, chronic respiratory disease in chickens, turkeys, game birds, pigeons and other wild birds. In adult birds, though infection rates are high, morbidity may be minimal. The mortality entirely to CRD is negligible, but it is important because it predisposes the birds to infection for other disease producing organisms.

Mode of Transmission:

M. gallisepticum is transmitted through eggs but organisms can also pass from bird to bird through nasal discharges and through droppings. It can also be transmitted by hands, feet and clothes of attendants of visitors. The route of infection is via the conjunctiva or upper respiratory tract with an incubation period of 6-10 days. Fomites appear to a significant factor in transmission between farms. Recovered birds remain infected for life; subsequent stress may

cause recurrence of disease.

Clinical Signs

The disease starts with Inappetance, sneezing, coughing, respiratory distress or gargling sounds during respiration. Eyes may show frothy exudates and conjunctivitis. The most common symptoms of infections in poultry are eye problems and inflammation around the face and cere. Other symptoms include open mouth breathing and gurgling throat sounds. Sometimes, when very chronic, the symptoms may hardly be seen. There may also be reduction in egg production to as much as 50%, 7-14 days postinfection.

Post- Mortem Findings

The most important pathological lesion is cloudy appearance of one or more air sacs. Usually the case become complicated and show cheesy material in the air sacs. Trachea and conjunctiva may be congested and there may be pericarditis and fibrinous covering on liver in cases complicated with *E. coli.*

Samples to be collected:

At necropsy, samples from active lung lesions should be collected for culture and histopathology. In case of cattle, sheep and goat these samples should be taken from the interface between consolidated and unconsolidated areas. Samples of pleural fluid, exudate from lung lesions, and regional lymph nodes should also be collected. Samples can be taken from live birds or fresh carcasses. From live and dead birds swabs may be taken from the oropharynx, oesophagus, trachea and cloaca. Exudates may be aspirated from the infraorbital sinuses and joints. Tissue samples for isolation should be collected aseptically, placed in a transport medium, kept cold, and shipped to the laboratory on wet ice. Samples should be frozen if they will not reach the laboratory within a few days; if necessary, samples can be stored at -20ºC for months with little apparent loss of *Mycoplasma*l viability.

Diagnosis

There are no clinical signs or gross or microscopic lesions which are pathognomonic in Mycoplasmosis. A definitive diagnosis can be made by isolating *Mycoplasma* from lung tissue and/or pleural fluid at necropsy. This organism has a branching, filamentous morphology in exudates, impression smears or tissue sections examined under the microscope. Biochemical, immunological and molecular tests can be used for identification of the culture. Biochemical tests are helpful in preliminary screening and as supporting tests for serology, but are unable to unequivocally identify the members of the *M. mycoides* cluster. Serological tests used to identify *Mycoplasma*s include growth inhibition, growth precipitation and immunofluorescence. Because members of the *M. mycoides* cluster are closely related and cross-react in these tests.

- **Isolation and Identification:**

Specimens are inoculated on to mycoplasma agar and into broth. Solid medium may help detection of slow growing mycoplasma colonies, which can be overgrown by saprophytes in broth. It may be necessary to make serial dilutions up to 10^{-3} for successful isolation. Inoculated plates are incubated at 37°C in sealed containers. Increased humidity and CO_2 tension in the atmosphere have been reported to enhance growth; these conditions may be obtained by the inclusion of damp paper or cotton wool, and by flushing the container with 5–10% CO_2 in nitrogen, by placing a lighted candle in the container, or by using a CO_2 incubator or suitable gas-generating system.

Broth medium should be examined daily for acidity, indicated by a change from red to orange or yellow in the indicator. Any observable growth is subcultured on to solid medium immediately. Even if no colour change occurs, subculture on to solid medium should be made after 7–10 days or earlier as the presence of an arginine-hydrolysing (alkali-producing) mycoplasma species may mask the acid colour change produced by MG. Mycoplasma colonies on solid medium can usually be recognised, although they may not have the typical 'fried egg' appearance. Bacterial colonies may appear on the first passage, but they are often more pigmented and fail to passage on mycoplasma media.

- **Serological Test:**

M. capripneumoniae and other members of the *M. mycoides* cluster cross-react in serological tests and share biochemical and genetic similarities, making specific identification of the organism difficult and time-consuming. Serological tests include complement fixation, latex agglutination, indirect hemagglutination and enzyme linked immunosorbent assays (ELISA). Serological tests are generally used on a herd basis and not for individual diagnosis. These tests do not identify all reactors, and cross-reactions occur with other species in the *M. mycoides* cluster. In addition, animals with acute CCPP rarely develop measurable titers before death.

- **Molecular Techniques:**

Polymerase chain reaction (PCR) assays are used to identify cultures of *M. capripneumoniae*, as well as to identify this organism directly in tissue samples.

Prevention and Control

The disease is most likely to enter a country by infected animals. Outbreaks can be eradicated with quarantines, movement controls, slaughter of infected and exposed animals and cleaning and disinfection of the premises. It is difficult to prevent infections caused by *Mycoplasma gallisepticum* because the disease is transmitted by eggs. It is necessary to protect birds against the complicating stress factors. Buying replacement stock from CRD free source greatly reduces the risk of spread. A very high standard of hygienic condition is of course, supremely important

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