

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Contagious Agalactia of Small Ruminants: Current Knowledge Concerning Epidemiology, Diagnosis and Control.

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ABSTRACT

Contagious agalactia is a highly infectious disease of sheep and goats which has been included in the B group of dangerous infections of OIE. *Mycoplasma agalactiae* is the major causal agent of the disease in both sheep and goats. The infection frequently occurs as an enzootic in different provinces of Iran, such as Khuzestan together with economic losses. It is usually manifested by mastitis, arthritis, keratoconjunctivitis, respiratory problems and mortality of young animals. The diagnosis is based on the conventional methods, such as cultivation and developed gene techniques and routine serological and also immunoenzymatic methods. In regions affected by an enzootic, treatment with antibiotics or vaccination of infected animals is used. In developed countries, there is a tendency to manage the disease in affected herds by gradual elimination of infected animals or even by killing them all at once. The main prerequisite for disease control is improvement of laboratory diagnosis with the use of monoclonal antibodies and gene amplification techniques and development of a new generation of efficient vaccines, e.g., sub-unit vaccines or those based on a synthetic antigen. This article reviews the epidemiology of the disease and prevention and control strategies of contagious agalactia.

Keywords: Sheep, Goat, *Mycoplasma agalactiae*, *Mycoplasma* species, Epidemiology

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INTRODUCTION

Contagious agalactia disease is known from two centuries ago. According Zavagli (1951), clinical symptoms of disease was first described by Methacza in Italy in 1816 and was named contagious agalactia by Brusasco in 1871. At present, the disease is prevalent in sheep and goats in countries where they are more dense (industrial) such as the Mediterranean and the Balkan Peninsula in Europe, West Asia and Southwest Asia and North, Central and Eastern Africa (1). In the former Czechoslovakia, the disease is registered for the first time in the group of sheep imported in early 1950 and it controlled with the slaughter of all infected animals. Then the disease has been eradicated in Czech Republic and this country is free from contagious agalactia disease now (38).

The economic importance

The morbidity rate of contagious agalactia disease is about 30-60% in a herd but the mortality rate of affected animals is low. However, in our study, no clinical signs of infection in flocks of sheep and goats in Ramshir (Khuzestan) have been reported 89.47% (57). Reduction or complete cessation of milk production and abortion in pregnant animals can bring great economic losses. A severe course of disease in flock may cause high mortality (up to 40-70%) in lambs and newborns. Mortality rate may reach 15-20% in adult animals. The disease is a serious problem in veterinary public health in countries where dairy sheep and goats imported food has an important role.

Etiology

Mycoplasma agalactiae, Causative agent of contagious agalactia, was isolated by Bridre and Donatien known as the second *Mycoplasma* species, in 1923. This *Mycoplasma* species was first named in 1931 by Wroblewski as *Anulomyces agalaxie* but according to a new taxonomy of *Mycoplasmas*, its name was changed by Freundt to *Mycoplasma agalactiae*, in 1957.

M. agalactiae, particularly in sheep, is considered as the classic etiologic agent of contagious agalactia disease (13). Other mycoplasma species are belonging to the "Mycooides cluster" may cause the same clinical signs and pathological conditions in small ruminants (13,52,60). This group includes *M. mycooides* subsp. *Mycooides* (large colony type (LC)), *M. capricolum* subsp. *Capricolum* and *M. mycooides* subsp. *capri*. A disease with similar clinical signs caused by *M. putrefaciens* in goats. present paper is focused on *M. agalactiae*.

M. agalactiae is a pleomorphic organism (124-250 nm) that like other mycoplasmas has relatively small genome (1×10^9 Da) and does not possess rigid cell wall but has flexible, triple-layered outer membrane. Mycoplasmas are susceptible to desiccation, heat, osmotic shock, detergents and disinfectants. However, they are resistant to antibiotics such as penicillin which interfere with the synthesis of bacterial cell walls. These organisms replicated by germination or dichotomy. Based on 5S rRNA sequence analyses, the mycoplasmas have been shown to be linked phylogenetically to Gram-positive bacteria which require enriched media containing animal protein, a sterol component and a source of DNA or adenine dinucleotide for growth (32). Their need for sterols, is reflected in their sensitivity to inhibition by digitonin. Newly isolated strains of *Mycoplasma* grows slowly on laboratory mediums but after adaptation to laboratory conditions, they can cultured easily in broth or agar mediums at 37°C (18, 33). *M. agalactiae* can not ferment glucose and arginine and is unable to hydrolysis urea. Most mycoplasmas are facultative anaerobes and some grow optimally in 5 to 10% CO₂, in a humid atmosphere at 37°C and have a 'fried-egg' appearance on agar mediums. These organisms are heterogeneous in terms of antigenic (12,63,67).

M. agalactiae is very sensitive to heat and drought conditions and inactivates at 60°C for 5 min and at 100°C for 1 min but survives at 8°C for more than 4 months and at room temperature for 1-2 weeks. This organism has long remained in the patient or carrier animals and disposed through the discharges especially milk and can survive infection. *M. agalactiae* is sensitive to the UV rays. The virulence of organism remains in -20°C for 8-9 months. Using disinfectants such as Chloramine, Potassium hydrochloride and Formaldehyde inactivated and destroyed mycoplasmas for 15-20 min (13,72).

Pathogenesis

Mycoplasmas are found on mucosal surfaces of the conjunctiva, nasal cavity, oropharynx and intestinal and genital tracts of animals and humans. Some species have tropisms for particular anatomical sites while others are found in many locations. Many mycoplasmas are non-pathogenic and constitute part of the normal flora of their host. Mycoplasmas survive for short periods in the environment (2,54,58).

Unlike many bacterial pathogens, mycoplasmas do not appear to produce specific toxins or invasins. However, some of the intrinsic metabolic functions of the organism appear to be important in its ability to produce disease, in addition to its ability to adhere to host cells and to evade the immune response. Production of H_2O_2 can induce toxic damage to host cells to which the organisms are adhering. Accordingly, adhesion to host cells is an essential attribute for pathogenicity but mycoplasmas do not invade the cells. Production of these soluble factors may be central to the pathogenicity of some strains as it has been shown that European strains of *M. mycoides* subsp. *mycoides* produce significantly less H_2O_2 than the more pathogenic African strains of this species (35,47). Some pathogenic species possess structures composed of unique adhesion proteins which promote attachment to mammalian cells. Mycoplasmas can adhere to neutrophils and macrophages and can also impair phagocytic functions. Variation in surface proteins is an important virulence attribute of *Mycoplasma* species as it allows the organism to rapidly adapt to the host environment and to evade the developing immune response. Such alterations in surface proteins have been demonstrated in many of the important animal pathogens including *M. mycoides* subsp. *Mycoides* and *M. bovis* (21,22). It is not known if the antigenic variation which occurs in these surface proteins is a regulated event or whether it is a random response due to selection pressure by the host immune response to surface antigens. An additional mechanism which may contribute to persistence of mycoplasmas in the host is the similarity between some mycoplasmal antigens and host tissue antigens. This may interfere with host recognition of mycoplasmal antigens during tissue invasion. In addition, it may predispose to the development of autoimmune disease if the host's immune response to mycoplasmal antigens damages tissues which share antigenic determinants with the invading pathogen (37).

Epidemiology

Contagious agalactia disease often prevalence enzootic in Khuzestan province of Iran and irreparable economic losses to the livestock population into it. The disease are quickly transported through contact of infected animals with healthy animals. Microorganisms spread occurs through of eye and nose discharges, milk, feces, urine and joint fluids and open wounds of infected animals or male animals with genitourinary system infection. The transmission of infection to teats possible occurs through infected hands or contaminant milking equipments. Newborn animals are usually infected by eating contaminated milk or colostrum. The sudden spread of the disease can be seen in the early spring, coinciding with the birth of lambs, in lactating ewes and in animals on pastures (37,41,59). At the start of summer, when infants are more susceptible to disease, the next step of spread is considering and increase the number of infected animals. The disease may happen during the next period of lactation in flocks or even years after. This condition can persist for several months in a herd unless appropriate measures are taken at the proper time (7,46). Early diagnosis of disease is important epidemiologically because after disease onset, infection agent spreads through milk for 12 months up to 8 years while the symptoms of disease are very mild or no longer visible (12,20,57).

There are no clinical signs of carrier animals in a herd can be a serious risk to the health of the herd. Infection agent concentrated in the reproductive system of these animals. Carrier state are less disrupted in male animals than females. The infection can be found in external ear canal of goats and were smaller in sheep (20). External ear canal is the perfect place for the organism because of lower general circulation, the immune system performance is not well (13,57). Studies have shown that other species of animals such as cows, camels and small ruminants can act as reservoirs of infection for *M. agalactiae* (53). Contagious agalactia antibodies remain in animal bodies against disease after infection in one area can have a lasting effect. The antibodies are present in the blood of goats over 8 years and in the sheep more than 3 years after onset of the disease (66).

Clinical signs

Contagious agalactia occurs in both sexes of sheep and goats. The incubation period of disease lasts from one week to two months. The incubation period depends on the pathogens virulence and host's

resistance. The clinical forms of infection includes an acute, subacute and chronic forms. Atypical individual form (50) and no clinical signs form occurrence of disease have also been reported (10). The first clinical signs of the disease include loss of appetite, vomiting, weakness and lethargy. The onset of disease along with mild fever that caused by the presence of mycoplasma in the blood of animals (mycoplasmaemia) and confirmation of organisms in the breast tissue, joints sheaths and conjunctival mucosa through the blood. Abortion may occur in pregnant animals. *M. agalactiae* are often isolated from vaginal and pulmonary lesions, despite that pneumonia can be seen only occasionally. At the beginning of the outbreak, chatarral or parenchymal breast swelling was seen. In these cases, breast tissue is hot, swollen and painful. Then breast is loosen and full of connective tissue and eventually cause atrophy. The milk of infected animals has become watery, salty, yellow or blue and two separate-phased. The upper phase is blue-gray and the lower phase is yellowish-green with clot. Gradually, the milk has pus and final cut production.

If the joints infected, the ankle joint would involve arthritis. Joints is swollen and painful and synovial fluid accumulates in them. In chronic cases, ankylosis may occur that cause lameness in host and in advanced form of the disease, the animal was unable to stand up and be grounded. If the organism is spread to the eye, firstly is observed keratoconjunctivitis and conjunctival hyperemia and then observed congestion of corneal surface. As the disease progresses, keratitis and loss of vision occurs caused by corneal angiogenesis. In some hosts, even if the injury occurred in the cornea, the disease subsides spontaneously after a short period (6,8,40,49,59).

Small ruminants infected by *M. mycoides* subsp. *Mycoides* (LC) usually becomes apparent arthritis, pleurisy, pneumonia and swelling of the conjunctiva. These mycoplasmas are everywhere and now found together in every continent, including areas where the prevalence of *M. agalactiae* are not normally. In goats, the disease usually occurs only sporadic. In such cases, infection remain for long periods in hosts and cause infection in other animals. *M. mycoides* such as *M. agalactiae* cause spread of disease, after birth, when the lamb eats infected colostrum or milk. Increased incidence of disease caused by septicemia, associated with arthritis or pneumonia and severe mortality in newborns (50). As yet, *M. mycoides* subsp. *Mycoides* (LC) has not been reported in sheep (47). This organism is present in different geographical regions of the world including Africa, Australia, Europe and the United States of America, but its occurrence is very low. The disease is more common in goats and will occur with symptoms such as fever, mastitis, septicemia and severe arthritis. Infected animals die within a short time. Symptoms of pneumonia is rarely found on necropsy examination (49). In goats, natural infection by *M. putrefaciens* have been reported only in a few cases but it has not reported in sheep (47). In cases where infection with *M. putrefaciens* occur, clinical signs include mastitis, agalactia and abortion with pregnant host deaths are occurring, but there is arthritis or nephropathy. In these herds, female animals and their infants often suffer from severe arthritis, but fever is found (21,64).

Diagnosis

Contagious agalactia diagnosis is performed based on epidemiological findings and clinical symptoms. When three typical symptoms include decreased milk production, mastitis and keratoconjunctivitis were observed in the herd, the disease can be diagnosed easily. In sheep and goats the symptoms can be seen immediately after birth, when signs of mastitis and reduced milk production occurs in lactating livestock. The most common form of symptoms of disease include mastitis and secretion of yellowish green milk. Ocular involvement is seen in only 50% of these hosts. Lameness is the most common symptom that remain for a long time in the herd and is seen in the male animals more than females. If only one set of symptoms seen among the flock, differential diagnosis of the disease is difficult. If host show only keratoconjunctivitis, may soon acquire full recovery and this signal is not detectable. If secondary infections occurs, deteriorated the situation even waste animal. In such cases, the mortality rate is about 20-15%. Clinical diagnosis should be confirmed by laboratory methods such as isolation and identification of the causative agent. The most suitable samples for laboratory diagnosis include the milk samples firstly and swabs of eye, vagina, nasal secretions, blood and urine secondly. For examination of autopsy, samples of breast tissue, lymph nodes, lung lesions and synovial fluids are properly. Mycoplasmas can be isolated from liver, kidney and spleen, but this sampling shall be performed during bacteremia. Also swabs taken from the rectum or external ear canal are suitable for laboratory diagnosis. For routine isolation of mycoplasmas, the samples should be inoculated into two broths and onto two plates of agar (41). Inoculated mycoplasmal mediums are incubated aerobically or in 5 to 10% CO₂, in a humid atmosphere at 37°C for up to 14 days. Colonies of *M. agalactiae* have a 'fried-egg' appearance on agar mediums.

Knowledge of the animal species and disease process will often suggest a particular mycoplasmal species. Precise identification of the species often requires sophisticated techniques and cultures are usually submitted to reference laboratories for final identification. The following methods are examples of the techniques that can be used to identify mycoplasmal species:

- Fluorescent antibody (FA) staining to identify *M. dispar* and ureaplasmas in bronchial epithelium of calves with pneumonia.
- FA technique (direct and indirect) for staining mycoplasmal colonies. This is the best method of recognizing mixed cultures and the method is used commonly with the avian mycoplasmas.
- Enzyme-linked immunoperoxidase used on frozen sections of porcine bronchial epithelium to detect *M. hyopneumoniae*.
- Agar gel diffusion tests using known antisera to identify prepared mycoplasmal antigen from broth cultures.
- ELISA and complement fixation test (CFT) for antigen identification using known antisera.
- PCR-based methods of identification have been developed for the identification of most species.
- Biochemical tests such as glucose fermentation, arginine hydrolysis, phosphatase activity and reduction of tetrazolium.
- Metabolic inhibition tests: essentially neutralization tests in which specific antisera prevent the utilization of a particular substrate.
- Growth inhibition tests using specific sera on filter paper discs (10).

A number of serological tests serological tests for the detection of antimycoplasmal antibodies are available and include:

- Rapid plate agglutination tests (coloured antigen) for screening poultry for the major mycoplasmal diseases.
- Haemagglutination-inhibition test for avian mycoplasmas.
- Indirect haemagglutination tests for *M. bovis* and *M. bovis genitalium*.
- Agar gel diffusion tests for avian mycoplasmas using known antigen.
- Complement fixation test for the control of contagious bovine pleuropneumonia and for screening pigs for enzootic pneumonia.
- ELISA techniques, usually indirect ELISAs, have been developed for detection of antibodies in many *Mycoplasma* infections of animals. Although the CFT was previously the principal test used for the purposes of international trade, ELISA tests are now prescribed tests for this purpose also.
- Latex agglutination test to detect antibodies to the mycoplasmas causing disease in goats.
- Dot-binding immunohistochemical testing (41).

The use of serological tests for diagnosing Mycoplasmas is easy, except in cases of cross-reactivity between *M. agalactiae* and *M. bovis* in animals that are closely related to each other.

Recently the genomic detection is possible using probes. These probes complementary to fragments of chromosomal DNA or 16s rRNA (8,23,66). At present, researchs to be focused on the use and development of the polymerase chain reaction (PCR) techniques that are more sensitive than other methods (24,67,69). PCR-based techniques have been developed both for direct detection of *Mycoplasma* organisms in clinical specimens and for identification of isolates obtained by culture. Multiplex PCR techniques for the detection and identification of several *Mycoplasma* species which affect a particular animal species are also available, for example, avian mycoplasmas or those affecting small ruminants (34). Several PCR procedures for the detection and differentiation of *M. bovis* are described, including real-time PCR (10,17,65). These assays may be used on clinical samples such as milk or lung tissue. Owing to the current inability to culture the haemotropic mycoplasmas *in vitro*, PCR assays are the best methods for diagnosis of infection with these species. Real-time PCR has been developed by a number of authors which can differentiate between the three haemotropic mycoplasmas known to infect cats (67,70).

Typing of *Mycoplasma* organisms is frequently carried out by enzymatic digestion of extracted DNA using *Sma*I, followed by pulsed-field gel electrophoresis (PFGE). However, digestion using *Bln*I and *Bam*HI was reported to give better discrimination of *M. synoviae* strains (67,70). Variable number tandem repeat (VNTR)

analysis and random amplified polymorphic DNA techniques may also be used. Isolates of *M. ovipneumoniae* with a number of different PFGE and RAPD patterns were identified within individual flocks in a UK study, suggesting introduction of strains by asymptomatic carrier animals (41,44). VNTR analysis detected greater variation in *M. agalactiae* isolates than PFGE typing and was considered a useful technique for outbreak tracing (72,55). Amplified fragment length polymorphism (AFLP) analysis has been used for typing *Mycoplasma* species also.

Treatment

The first drugs used in the treatment of contagious agalactia of sheep and goats were arsenic compounds and sodium salts. In countries with ongoing spread of the disease, conventional treatment done by antibiotics such as Tetracyclines, Macrolides, Tiamulin and Fluoroquinolones (5,14,28,64). For the treatment of contagious agalactia, the use of systemic antibiotics is recommended but in certain conditions such as chronic mastitis, intramammary use of antibiotics in non-lactating female animals is recommended (27). If the antibiotics doses are not careful or not use antibiotics for the full course of therapy, infectious agents released into the environment and caused resistant strains.

Control and prevention

Vaccination of sheep and goats against contagious agalactia done by live attenuated or inactivated vaccines (16,29,30,45,67). At present, use of agalactia inactivated vaccine which is contains three native strains and saponin and manufactured by Razi institute, is as the best way to prevent of disease in Iran. Strains such as *M. agalactiae* and *M. mycoides* subsp. *Mycoides* are used in this vaccine production. Although live attenuated vaccines are more effective than inactivated vaccines but use of these vaccines is not permitted in all countries where the contagious agalactia is prevalence and is possible due to the release of disease agents in the environment. If infected animals vaccinated with a live vaccine, will resume their normal feeding and articular lesions have subsided, but pathogens are excreted through milk for a few months. If vaccination is done in healthy animals, the infection does not spread and do not cause clinical signs, it may appears just a temporary infection in the breast. Inactivated vaccines develops a weak immune response in animals. However, in field conditions, it would be the type of vaccine used. While the use of vaccines against contagious agalactia disease is spreading, but their effectiveness is disputed generally (31,50). Economic losses caused by the disease is remarkable in the absence of treatment or vaccination (2,54,58). Vaccination against contagious agalactia is appropriate in areas where the disease is prevalent in enzootic form, especially in areas with poor economic and social status. For these countries, providing a new generation of effective vaccines against contagious agalactia is welcomed.

To protect herds of sheep and goats against contagious agalactia disease, is essential to respect the veterinary and sanitary regulations and proper animal husbandry practices. It seems that the most effective measure to prevent the spread of contagious agalactia in disease-free areas is destroying all diseased, clinically affected and susceptible animals in the herd. However, implementation of such a program especially in less developed countries may be difficult because of the economic and social considerations.

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