Review Article
CONTAGIOUS AGALACTIA OF SHEEP AND GOATS. A REVIEW

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Abstract

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Contagious agalactia is a highly infectious disease of sheep and goats which has been included in the List B of dangerous infections issued by the International Office International des Epizooties. The major causal agent of the disease in both sheep and goats is *Mycoplasma agalactiae* and, in goats, the disease can also be caused by *Mycoplasma mycoides* subsp. *mycoides large colony type* (LC), *Mycoplasma capricolum* subsp. *capricolum* and *Mycoplasma putrefaciens.* The infection frequently occurs as an enzootic. In lactating female animals, it is usually manifested by mastitis. Males, young animals and non-lactating females suffer from arthritis, keratoconjunctivitis and respiratory problems. Young animals may often die. The diagnosis is based on the isolation and identification of the infectious agent by conventional methods, such as cultivation, growth inhibition test, epi-immunofluorescence test, etc., as well as on recently developed techniques such as dot-immunobinding test, gene probes and polymerase chain reaction. The routine serological method is the complement-fixation test and, recently, also immunoenzymatic methods. In regions affected by an enzootic, therapy with antibiotics or vaccination of infected animals are used. In economically and socially 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 a quick and exact laboratory diagnosis of each mycoplasma species, with a particular emphasis placed on the identification of species included in the "mycoides cluster" group. This is expected to improve greatly with the use of monoclonal antibodies and gene amplification techniques. The development of a new generation of efficient vaccines, e.g., sub-unit vaccines or those based on a synthetic antigen, is considered to be a further essential step in contagious agalactia eradication.

Sheep, goats, contagious agalactia, Mycoplasma agalactiae, Mycoplasma species

History

Contagious agalactia of sheep and goats has been known for about two centuries. As reported by Zavagli (1951), the clinical disease was first described by Metaxa in Italy in 1816 and was given the name contagious agalactia by Brusasco in 1871. At present it occurs in most of the countries with intensive production of sheep and goats, i.e., in the Mediterranean region and the Balkan peninsula in Europe, in western Asia and in northern, central and eastern Africa (Erdag 1989; Al-Zeftawi 1979; Belaid et al. 1990; Sarris 1996; Lambert 1987; Bergonier 1997; Nicholas 1995; Da Massa et al. 1992; Damdinsuren 1989; Ismail 1993; Egwu et al. 1999; Kusiluka et al. 2000). In the former Czechoslovakia, the disease was last recorded in the early 1950s in a group of imported sheep (Koppel 1982). The situation was managed by killing all the infected animals. Since then the Czech Republic territory has been free from this disease.

Economic importance

Although contagious agalactia does not show high mortality, morbidity from this disease in a herd may be 30-60 %. A reduction, in or complete cessation of, milk production or

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abortions in pregnant animals may bring about economic losses. A severe course of the disease in herds may result in the death of lambs and kids (up to 40-70%). In rearing units, the losses may reach to 15-20%. In countries where sheep and goat dairy products play important roles as food components and import commodities, contagious agalactia is a serious veterinary public health problem.

Aetiology

The causal agent of the disease, *Mycoplasma agalactiae (M. agalactiae)*, was isolated by Bridre and Donatien in 1923 as the second known mycoplasma species. It was originally termed *Anulomyces agalaxie* by Wroblewski in 1931 but in 1957, in accordance with the newly established taxonomy of mycoplasmas, its name was changed to *M. agalactiae* by Freundt.

M. agalactiae is regarded, particularly in sheep, as the "classical" aetiological agent of contagious agalactia (Bergonier 1997). However, similar clinical and pathological features can be produced in small ruminants by other mycoplasma species (Sarris 1996; Bergonier 1997; Nicholas 1996) included in the "mycoides cluster". This group contains *Mycoplasma mycoides* subsp. *mycoides* large colony type (LC), *Mycoplasma capricolum* subsp. *capricolum* and *Mycoplasma mycoides* subsp. *capri*. In goats, a disease with nearly identical clinical manifestations is caused by *Mycoplasma putrefaciens.*

In this study, special attention will be paid to *M. agalactiae.* This is a polymorphic bacterium 124-250 nm in size. Similarly to other mycoplasmas, it has a very small genome (1×10^9 Da) and no cell wall but only the plasma membrane. As all mycoplasma species, it is resistant to penicillin and its analogues but sensitive to osmotic shock and the effect of detergents. It multiplies by budding or binary division, is Gram-negative and grows well on special liquid and solid media with the addition of sterols. Its growth is promoted by aerobic conditions (Freundt 1957). The cells are sensitive to digitonin. Freshly isolated strains show slow growth but, after adaptation to laboratory conditions, they can be cultivated in the majority of liquid or solid media at 37 °C (Cottew 1985; Freundt 1983; Lambert 1987). *M. agalactiae* cells do not ferment glucose and hydrolyse neither arginin nor urea. Cultivation on solid media requires a humid atmosphere with 5 % CO_2 and the colonies produced have a typical fried-egg appearance. For a long time *M. agalactiae* was considered to be an antigenically uniform species (Al – Aubaidi and Dardiri 1974); its antigenic heterogeneity has been reported only recently (Bergonier 1996 ad; Solsona et al. 1996; Tola et al. 1996a).

M. agalactiae is sensitive to increased temperature. At 60 °C it is inactivated in 5 min and at 100 °C in a minute. It survives up to 4 months at 8 °C and for one or two weeks at room temperature. At –20 °C it remains virulent for 8 to 9 months. It is also sensitive to ultraviolet radiation. This organism is inactivated and dies quickly in a putrescent environment. It is destroyed within 15 to 20 min by commonly used concentrations of disinfectants such as chloramine, potassium hydrochloride and formalin (Bergonier et al. 1997; Tsaknakis et al. 1992).

Pathogenesis

Under natural conditions, the most common means of entry for *M. agalactiae* are oral, respiratory or mammary routes. In animals infected orally, it is assumed that the primary site of adhesion and subsequent invasion is the small intestine (Zavagli 1951; Hasso et al. 1993). This assumption was confirmed in studies in which *M. agalactiae* was isolated from rectal swabs and the small intestine of animals after experimental infection (Hasso et al. 1993). In natural conditions it is difficult to determine if the infection has been acquired via

the respiratory route but experimental infection with *M. agalactiae* has been successful (Damdinsuren 1989; Da Massa et al. 1992). Infection and subsequent colonisation of the mammary gland with *M. agalactiae* is thought to be a result of incorrect milking techniques or defective milking equipment (Da Masssa et al. 1987; 1992; Kinde et al. 1994).

Infected animals develop bacteraemia accompanied by fever. The infectious agent is transferred by circulating blood to the target organs, i.e., the mammary gland, eyes, lymph nodes, joints and tendons, in which inflammatory changes are produced. Pregnant animals may abort due to inflammation of the uterus or may give birth to non-viable offspring. Male animals may show inflammatory changes in the testes.

Epidemiology

The disease is rapidly spread by contact between infected and healthy animals. Dissemination of the microorganisms into the environment occurs by means of ocular and nasal discharge, milk, faeces, urine and excretions from open joint lesions or the male genitourinary tract. The infection can also be transmitted via teats contaminated by milking equipment or the hands of milkers. Young animals are most commonly infected when suckling contaminated colostrum or milk. A flare-up of the disease is observed early in spring when young animals are born, females are in full lactation and the animals graze on pastures (Lambert 1987; Real et al. 1994; Kinde et al. 1994). A further increase in the number of infected animals is recorded at the beginning of summer when young animals are very sensitive to infection. The disease in a herd can persist for several months unless adequate measures are taken in time. The disease can often recur during the next lactation or even in several following years (Belaid et al. 1990; Levisohn et al. 1991).

In terms of epidemiology it is important to recognise that, following the disease, the causal agent is excreted with milk for a minimum of 12 months and a maximum 8 years by which time clinical signs are very mild or no longer present (Da Massa and Brooks 1991; Bergonier et al. 1996d).

The presence of asymptomatic carriers in a herd appears to be a serious health risk. These animals carry the infectious agent in their genital tracts; the carrier state is less obvious in males than females. In goats and less frequently in sheep, however, the infection can be present in the external auditory canal. This rather exceptional site is advantageous for the microorganism because the host's defence mechanisms cannot well operate here (Bergonier et al. 1997).

It has been shown that other animal species, such as cattle, camels or small wild ruminants, can function as infection reservoirs for mycoplasmas (Perrin et al. 1994).

It appeared from the studies on the persistence of post-infection antibodies against contagious agalactia that antibodies are present in goats for up to 8 years and in sheep up to 3 years after the manifestation of clinical disease (Tola et al. 1994).

Clinical signs

Sheep and goats of both sexes can be infected by contagious agalactia. The incubation period may last from one week to two months. Its duration is related to the degree of virulence of the infectious agent and the overall resistance of the host. Clinical disease can be manifested in an acute, a subacute or a chronic form. A sporadic occurrence of atypical (Nicolet 1994) or asymptomatic forms has also been reported (Bergonier 1996 abc).

Anorexia, lethargy and unwillingness to follow the herd are the first clinical signs. An onset of a brief febrile syndrome due to mycoplasmaemia precedes colonisation of the mammary gland, joint lining and conjunction mucosa by the infectious agent. Pregnant

animals may abort. *M. agalactiae* has been isolated also from vulvovaginal and pulmonary lesions but pneumonia has been observed only occasionally.

At the beginning, the udder shows catarrhal or parenchymatous mastitis. It is hot, swollen and tender. Later it becomes flaccid, filled with connective tissue and, eventually, atrophy develops. Milk turns into a yellowish or bluish fluid that has a salty taste. It can have a watery consistence and, when allowed to stand, separates into an upper, grayish-blue and a lower, yellowish-green layer with clots. Milk gradually becomes purulent and, at the final stage, its production ceases.

When joints are affected, arthritis usually involves the carpus or tarsus. The joint is swollen and painful, with accumulation of synovial fluid. In chronic cases, ankylosis may develop, which makes the animal limp or prevents it from standing.

In ocular localisation, the first sign of keratoconjunctivitis is congestion of the conjunctiva and, later, vascularisation of the corneal surface. This may be followed by kerato-conjunctivitis, keratitis and the loss of sight due to vascularisation of the cornea. Some animals heal spontaneously after a short time, even though they have undergone ulceration of the cornea (Bar-Moshe et al. 1984; Benkirane and Amghar 1990; Kwantes and Harby 1995; Mega et al. 1993; Real et al. 1994).

Infection of small ruminants with *M. mycoides* subsp. *mycoides* LC is usually manifested as arthritis, pleurisy, pneumonia and keratoconjunctivitis. This mycoplasma species is ubiquitous and is present on all continents including the regions where *M. agalactiae* is not common or occurs only very rarely. When goats are affected, only sporadic cases are usually involved. However, if present, the infection may persist for some time and be a source of infection for the other animals in a herd. Similarly to infection with *M. agalactiae*, the potential for spreading the disease increases after parturition when kids suckle infected colostrum or milk. This may result in septicaemia accompanied by arthritis or pneumonia and in high mortality of the kids (Nicolet 1994). *M. mycoides* subsp. *mycoides* LC is almost never found in sheep.

M. capricolum subsp. *capricolum* is present in geographically different parts of the world (Africa, Australia, Europe and USA) but its occurrence is very low. It affects goats more often than sheep and the disease is manifested by fever, septicaemia, mastitis and severe arthritis. The diseased animals may die in a short period of time. Postmortem examination frequently reveals signs of pneumonia (Mega et al. 1993).

Natural infection with *M. putrefaciens* has been reported only in goats and only in a few cases. Not a single case of infection has been described in sheep. In some conditions associated with *M. putrefaciens* isolation, clinical signs were mastitis and agalactia but not arthritis or ocular lesions; other conditions included, in addition to mastitis and agalactia, abortions with subsequent death in pregnant females. In the herds affected by this form of the disease, mothers and their kids also suffered from severe arthritis; fever, however, was not observed (Da Massa et al. 1992; Stipkovits et al. 1984).

Diagnosis

The diagnosis of contagious agalactia is established on the basis of epidemiological findings and the presence of clinical signs. It is relatively easy when all three typical clinical features are observed in the herd, i.e., loss of milk production and mastitis, keratoconjunctivitis and articular lesions. In both sheep and goats, the signs become manifested shortly after parturition when lactating animals develop mastitis. The most common form is a mastitis accompanied by yellowish-green milk secretion. Ocular involvement may be found in only about 50% of cases. Lameness, which is common and may persist for a long time, is observed more frequently in males than females. However, if only one form of the disease is present, it may be very difficult to make a clinical diagnosis. If the

animals show only keratoconjunctivitis, they may recover completely. If, however, they get a secondary bacterial infection, they may even die. Mortality in such cases is 15-20 %.

The clinical diagnosis should be confirmed by laboratory examination, i.e., isolation and identification of the infectious agent. The best material for analysis is milk, then ocular, vaginal or nasal swabs, articular exudates, blood and urine. For post-mortem examination, samples are collected from the mammary gland and regional lymph nodes, pulmonary lesions and articular exudates. Mycoplasmas can also be isolated from liver, kidney and spleen tissue but the samples must be collected at the stage of bacteraemia. Swabs taken from the rectum or external auditory canal also provide biological materials suitable for examination. Cultivation is carried out in liquid or on solid media which support mycoplasma growth (Lambert 1987). *M. agalactiae* produces colonies with centres and a phenomenon called "film and spots". The use of biochemical tests for mycoplasma identification is time consuming and gives results difficult to interpret (Lambert 1987).

Serological methods for identification of the causal agent include growth-inhibition and metabolism-inhibition tests and epi-immunofluorescence and peroxidase tests. Advantages and disadvantages of these tests have been discussed by Lambert (1987). A more recent identification method is based on the dot-immunobinding test. Serological identification of *M. agalactiae* and *M. putrefaciens* is easy except in the cases where there is a cross-reactivity between *M. agalactiae* and *M. bovis*, i.e., species that are closely related. However, difficulties may arise with distinguishing between *M. mycoides* subsp. *mycoides* LC and *M. capricolum* subsp. *capricolum*; these species are both included in the "mycoides cluster" that covers the whole range of mycoplasma species infectious for ruminants. These species show great similarity in genetic, protein and antigenic characteristics but cause different clinical diseases (Bergonier et al. 1997). The difficulties in identifying members of the "mycoides cluster" can be overcome by the use of monoclonal antibodies against individual mycoplasma species or by means of gene amplification techniques (Bergonier et al. 1996 abc). Recently, genomic detection has been made possible by the development of gene probes. The probes are usually complementary to segments of chromosomal DNA or 16S ribosomal RNA (rRNA) (Mattson et al. 1991; Dedieu et al. 1992; Tola et al. 1994). Although their specificity is high and they have facilitated great progress in the diagnosis of mycoplasma infections, research efforts have recently been focused on the development of polymerase chain reaction (PCR) techniques that seem to be even more sensitive.

Several systems have been proposed for the detection of *M. agalactiae, M. mycoides* subsp. *mycoides* LC or *M. capricolum* subsp. *capricolum*, some of them combining an amplification step with two pairs of different primers and an identification step based on the analysis of restriction profiles of PCR products (Dedieu et al. 1995; Tola et al. 1996ab; Tola et al. 1997). Other PCR techniques are based on 16S rRNA (Bergonier et al. 1996b; Chavez et al. 1995) or on amplification of segments of the uvrC gene (Subramaniam et al. 1998). The most recent approach described by Greco et al. (2001) is the multiplex-PCR. However, PCR methods carry a high risk of false-positive reactions due to contamination of the product. For this reason, they are not, at the present time, considered suitable for routine diagnostic procedures in field laboratories. Inter- and intra-species genetic variation can also be evaluated by pulsed – field gel electrophoresis (Tola et al. 1996a; Tola et al. 1999a).

Serological diagnosis in the past was based on several techniques such as immuno-fluorescence, slide agglutination, tube agglutination, growth-inhibition and immunodiffusion tests, etc. (Lambert 1987; Le Goff a Perreau 1984; Tsaknakis et al. 1992). At present, antibodies in blood serum or milk are detected by means of the complement-fixation test or ELISA techniques. The complement-fixation test was routinely used to diagnose *M. agalactiae* mainly in the 1970s, while in the 1980s it was preferred for

the diagnosis of *M. mycoides* subsp. *mycoides* LC and *M. capricolum* subsp. *capricolum*. The immuno-enzymatic techniques were established in the diagnosis of contagious agalactia in 1982 and were used preferably for *M. agalactiae* and *M. mycoides* subsp. *mycoides* LC. In the following years, a trivalent test for all three above mentioned species was developed (Belaid et al. 1990; Lambert et al. 1998; Schaeren and Nicolet 1982). Serological methods are generally very useful in supporting the diagnosis based on laboratory examination and are of value particularly in epidemiological investigations. However, they can never replace the isolation and identification of an infectious agent by the standard methods (Nicolet 1994).

Differential diagnosis is necessary in order to distinguish mastitis and keratoconjunctivitis of mycoplasmal origin from the conditions caused by other microorganisms, to eliminate brucellosis, listeriosis and lentivirus infections and abortions due to *Chlamydia, Salmonella* or *Campylobacter* species.

Therapy

The first drugs used in attempts at treating contagious agalactia of sheep and goats were arsenic compounds, particularly sodium and zinc salts of acetarsol. The current therapy in countries with persistent incidence of the disease is based solely on antibiotics, namely, tetracycline, macrolide, florfenicol, tiamulin and fluoroquinolones (Stipkovits et al. 1984; Bagherwal and Sisodia 1991; Bhaumik et al. 1990; Fadda et al. 1995). The majority of authors have recommend systemic administration of antibiotics but, in certain conditions such as chronic mastitis, intramammary application has been described in females that have dried off (Esnal et al. 1994). However, many authors feel that bacteriological treatment is an illusory objective. If the therapeutic dose is not exactly defined and the relevant antibiotic is not administered for a sufficiently long period, the resulting effect may be very poor or none at all. The causal agents continue to be shed into the environment and there is a possibility that resistant strains will develop.

Prophylaxis

Vaccination strategies against contagious agalactia of sheep and goats are based on both live attenuated or inactivated vaccines (Foggie et al. 1971a; Foggie et al. 1971b; Leon–Vizcaino et al. 1995; Sarris et al. 1989; Buonavoglia et al. 1998; Tola et al. 1999b) and the efficacy of this approach has been repeatedly evaluated (Lambert and Cabasse 1989; Hasso et al. 1993). Antigens for vaccination have been prepared mainly from *M. agalactiae* and *M. mycoides* subsp. *mycoides* LC. Live vaccines from attenuated *M. agalactiae* cultures are more effective that inactivated vaccines, but their use is not permitted in all the countries that are affected by contagious agalactia. If a live vaccine is applied to infected animals, normal lactation is resumed and articular lesions subside, but the pathogens continue to be excreted with milk for several months. If healthy animals are vaccinated preventively, there is neither generalised infection nor the development of clinical signs, but a temporary infection of the udder may appear. Inactivated vaccines are free from these disadvantages but the immune response they produce is so low that, in field conditions, they are utilised only occasionally.

While the use of conventional vaccines against contagious agalactia is still in progress, their efficacy is widely disputed (Food and Agriculture Organisation 1992; Nicolet 1994). Vaccination is considered to be appropriate in regions affected by enzootics and particularly in areas with a low social and economic standard where implementation of more effective, radical measures will be difficult. It is obvious that, for these countries, the development of new-generation vaccines against contagious agalactia would be most welcome.

Protective measures

The optimal measure to protect sheep and goat herds from contagious agalactia is consistent adherence to veterinary, hygienic and husbandry measures and regular mechanical cleaning and disinfection of barns. If the infection enters a territory so far free from contagious agalactia, the most effective measure to prevent its spreading appears to be complete eradication of all affected animals, animals suspected to have the infection and susceptible animals in the herd. However, because of the economic and social impact of such a programme, the implementation of radical approaches may be difficult, particularly in less developed countries.

Nakažlivá agalakcie ovcí a koz

Nakažlivá agalakcie je vysoce kontagiózní onemocnění ovcí a koz, které je zahrnuto v seznamu B nebezpečných nákaz Mezinárodního úřadu pro nákazy. Za klasického původce této nákazy je u obou druhů zvířat považováno *Mycoplasma agalactiae*, u koz se pak ještě připojují: *Mycoplasma mycoides* subsp. mycoides LC, *Mycoplasma capricolum* subsp. *capricolum* a *Mycoplasma putrefaciens*. Výskyt nákazy má většinou enzootický charakter. U laktujících samic probíhá onemocnění za příznaků mastitid. Samci, mláďata a samice, mimo období laktace, trpí artritidami, keratokonjunktivitidami a respiračními potížemi. U mláďat dochází často k úhynům. Diagnostika onemocnění je v současné době založena na izolaci a identifikaci původce jednak klasickými metodami (kultivace, růstově inhibiční test, epi-imunofluorescenční test aj.), ale i pomocí nových technik (dot imunobinding test, genové sondy, polymerázové řetězové reakce). Tradiční serologickou metodou je komplement – fixační test, nověji jsou využívány imunoenzymatické metody. Terapeuticky se v některých enzozoticky zamořených oblastech podávají antibiotika nebo se provádí vakcinace. V sociálně a ekonomicky vyspělých státech je snahou tlumit onemocnění postupnou eliminací postižených zvířat z chovů nebo jejich radikální likvidací. Hlavním předpokladem úspěšného zvládnutí této nákazy v chovech je rychlá a přesná laboratorní diagnostika jednotlivých mykoplazmových druhů a především pak těch, které patří do skupiny „mycoides cluster." Velkou nadějí do budoucna je proto rozšíření spektra detekčních metod využívajících monoklonální protilátky a genově amplifikační techniky. Jako zcela nezbytný se dále jeví vývoj účinných vakcín nové generace – například subjednotkových nebo založených na přípravě syntetického antigenu.

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