
Розділ 3. Ветеринарна мікробіологія та вірусологія

NOCARDIA MASTITIS

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Nocardia genus is recognized worldwide as a cause of disease in human beings and animals, particularly mammals and birds. In cows they can cause hepatitis, endocarditis, pneumonia and abortion. *Nocardia* spp. are an uncommon cause of mastitis, and outbreaks have typically been reported in dairy farms with poor hygienic and management conditions, and on farms where inadequate intramammary antibiotic therapy is conducting.

Etiology. Pathogens belonging to this family are named after Actinomycetaceae Nocard, which exposes them to different kinds of Actinomyces from the same family for its anaerobic growth and partial resistance to acids.

Nocardia type includes many strains, of which only one part is considered pathogenic. *Nocardia asteroides* is in the spotlight. *Nocardia* are ubiquitous bacteria, whose presence can be proved in the soil from pastures, meadows, surface water, dirt on the feed. Also present in the vicinity of infected animals. In microscopic preparations *Nocardia asteroides* is observed as grampositive, branched, partially granular or as a long string, coccoid wand.. The causative agent is resistant to alcohol. After a 48-hour incubation at 37°C under aerobic conditions, *N.asteroides* growing at 5% sheep blood agar as white-gray, round, oval and bizarre colony diameter from 0.5 to 1.5 mm. Possible by the appearance of colonies that are smooth and shiny. Striking a raised center colony that was almost completely submerged in the agar and its crumbly consistency to sand. On solid media with the addition of blood there is no hemolysis. However, reveals a pronounced susceptibility to staining. Enzymatic activity of nocardia is, in general, quite poor on average requires three to four weeks of incubation at 37°C. To test the pathogenicity of *Nocardia asteroides* are used primarily rabbits and guinea pigs are less suitable. Mice are not suitable for these tests. We can see a very different pathogenic nocardia strains. While, for example. *N.leishmanii* non-pathogenic for guinea pigs, *N.asteroides* pathogenicity for cattle is parallel to pathogenicity for guinea pig. *Nocardia* are thermo-stable so heat of 70°C for 30 minutes does not kill them. Pasteurization of milk has no effect here. *N.asteroides* is largely resistant to drugs. However, chloramphenicol, streptomycin, neomycin, sulphathiazole, izonicotin acid-hydrazide, cycloserine inhibit the growth of *Nocardia in vitro*.

Pathogenesis. As microorganisms are facultative pathogens for udder, nocardia require the presence of predisposing factors that could reach the mammary gland, settled in it and caused disease.

As routes of infection of the udder are cited:

- Intracisternal;
- hematogenic;
- percutaneous.

It should not be excluded penetration of agent through the skin of the udder injuries to the inside of the body. The possibility of infection through the teat canal is directly related to the hygiene conditions in the facilities where animals are kept, milking and udder hygiene and specific factors of the organism.

As the key points that may favor infection stand out:

- Lack of cleanliness and disinfection of housing sites;
- The wetland pastures or enclosures for livestock in the open;
- Lack of cleanliness and disinfection of equipment for milking, especially if the animals are kept in the open (open area);
- Blind milking;
- Damage to tissue of the udder by mechanic or infectious insults.

Special attention must be paid to intracisternal application of antibiotics. Besides the well-known irritation of the mammary gland caused by this kind of treatment, the nocardia infection is possible in cows whose udder is treated intracisternal with penicillin. These factors are conducive to infection and this can be explained by the fact that the nocardia in penicillin preparations can survive at least 2 days and in certain circumstances in these preparations even reproduce. In the case of incorrect intracisternal administration and non-compliance of prescribed norms of asepsis in operation we must consider possibility of transmitting infection. This applies to the application of antibiotics in the dry period, and increasing of estrogen level that contribute to the growth of microorganisms. Also increased milk production post partum increases the risk of infection. Intracisternal experimental udder infection with *N.asteroides* flows analogous to spontaneous infection, and on the basis of localization and spread of pathological processes in the udder indicates that intracisternal route of infection in common.

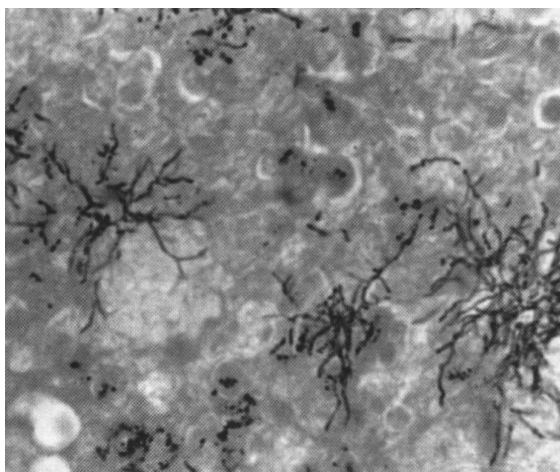
Clinical picture and course of disease. In cows with clinical mastitis in about 10% (up to 30%) of cases there are acute inflammation of the udder, or at least one area, that are resistant to all standard treatments. Udder was diffuse, markedly increased, due to strong tension of painful tissue. Lymph nodes of the udder were raised due to increased volume. Secret from infected udder has the appearance of watery-milk looks similar to serum, contains flakes, and in a short period of time becomes slimy purulent. May be expelled from the teat canal with great effort in very low amount. The effect of oxytocin stops. General health status changes. Besides loss of appetite, drastic reduction in milk production is evident and body temperature up to 42 °C, and the conspicuous loss of body weight. The result is a need for economic evaluation of the sick animals.

Chronic mastitis may be observed in a large percentage (60 %). Clinical finding is rough thickening of tissue in the form of large nodes, which develop in the abscess tissue changes. Secrete a thick, purulent discharge with mushy odor. Chronic mastitis without pre-acute phase is characterized with less conspicuous gland tissue thickening, hardening lobular, atypical watery-milky discharge. Enlarge of the affected area leads to reduced milk production and the tendency of dry udder. Depending on the spread of infectious agents it may lead to clinical latent infection in most of the cows in the herd, where can be seen slightly altered secretion. In clinical mastitis udder is altered and in 75 % of cases we can prove the presence of pathogens in secretions. Dry cows and heifers may have clinically latent infection or chronic diseases. In mixed infections clinical picture may differ.

Pathomorphology. Findings on the udder in clinical form of mastitis can be described with:

- subcutaneous edema;
- easy cutting of tissue;
- clearly marked areas of differ tissue.

In chronic form of disease most important findings are granuloma in areas of softening tissue.



Picture 1 Findings on *Nocardia* in granuloma

Diagnosis. Diagnosis relies on the methodological assessment of clinical, cytological, bacteriological and pathomorphological findings for each animal. It can be applied to the diagnosis of the general situation in the herd in case of possible outbreaks. Although clinically can be set only suspect diagnosis, because the individual animals can be spotted in largely non-specific symptoms, significant entry points are:

- Frequent occurrence of disease in certain groups of cows (particularly susceptible);
- Clinical presentation and course of disease;
- Frequent occurrence of resistance to therapy.

In any case, the individual animals and all the cows in the herd, it is necessary to set up and document the precise clinical and palpatory findings. Cytological examination of samples of secretion in different stages of *Nocardia* mastitis did not give results that differ from the results of mastitis with different genesis. About 75 % of samples were bacteriologically positive for nocardia and with increased number of somatic cells. Bacteriological diagnosis applied by cultivation on agar with 5 % sheep blood, microscopy of smears stained by modified Ziehl-Neelsen and Gram stain method and experiments on laboratory animals (guinea pig). The finding on granulomatous character-*Nocardia* mastitis pathogens in the tissue is supported by histopathologic diagnosis and complements bacterial pathogens. As the optimal method for detection of pathogens in the tissue proved to be the method of silver plating by Gomori, modified by Grocottu, and staining by a modified Ziehl-Neelsen method. Immunological method for detection of nocardia infection has not proved yet.

Forecast. Since in case of acute course of the disease we must take into account not only the loss of quarter, but also the loss of animals, it is necessary to make timely economic assessment of the treatment of animals. In chronic or latent infection infected animals can produce milk.

Prophylaxis. Prophylactic measures should primarily be directed to:

- Prevent contact with the cause of mastitis in animals as much as possible;
- Implementation of an effective decontamination agent;
- Limit the removal of a predisposition of the body, particularly the udder, the occurrence of the disease;
- Targeted use of diagnostic measures for health care provided by Epidemic.

In the framework of protective measures are of particular importance, especially the protection of animals from contaminated food and material used as litter. In animals on pasture, primarily in the swampy terrain, it is necessary to take account of the hygiene of the pasture, the place where animals are kept and the places where her husband is. Central place in the framework of these measures takes milking hygiene. Although in the presence of other causes of mastitis does not exclude antibiotic therapy, which indicates that

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the application of this therapy is only in a very clear indication of the presence of these pathogens and the strict regime of application. First of all, it is necessary to thoroughly clean and disinfect premises and equipment for her husband. As a suitable disinfectant shall be dissolved chlorine and peracetic acid for areas in which the husband is a formalin solution (5%) for areas in which animals are kept. For dipping teats as disinfectants in use are based on amphotenzide or peracetic acid. Fine disinfecting solutions used alcohol or paint acridine or quaternary ammonium compounds. With prophylactic standpoint, in the case of territorial threat of nokardija next review herds and affected animals are essential and preventative care of animals that are introduced into the farm.

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НОКАРДИЯ- МАСТИТ

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У статті розглядаються проблеми щодо етіології, патогенезу, діагностики та профілактики бактеріальної інфекції вим'я – нокардія-мастит. Під *Nocardia* відомий у всьому світі. Бактерії *Nocardia* викликають хвороби у людей і тварин, зокрема у свавців та птиці. У корів вони спричиняють гепатит, ендокардит, пневмонію. *Nocardia* spp. Рідко спричиняють мастит, але в деяких господарствах при певних умовах такі інфекції отримують епізоотичне розповсюдження.

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ВИВЧЕННЯ БІОЛОГІЧНИХ ВЛАСТИВОСТЕЙ ШТАМУ *PASTEURELLA MULTOCIDA* «СМОЛ» ЗА УМОВИ S-R-ДИСОЦІАЦІЇ

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При виробництві ветеринарних імунобіологічних засобів (вакцин, сироваток, діагностиків, та інш.) використовують патогенні штами мікроорганізмів. Для більшості вірулентних бактерій характерним є зростання в S-формі. Однією з проблем, що виникає в процесі роботи з виробничими штамами бактерій є дисоціація. Відомо, що в процесі дисоціації бактеріальної культури одночасно відбуваються зміни морфології колоній, біохімічних, антигенних, патогенних властивостей бактерій, їх стійкість до фізичних та хімічних факторів зовнішнього середовища[1, 2].

У зв'язку з тим, метою роботи було вивчення біологічних властивостей штаму *Pasteurella multocida* «Смол», якому властиві спонтанні S-R- дисоціації, за культурально-морфологічними, біохімічними властивостями, ступнем вірулентності, рівнем антибіотикорезистентності

Матеріали і методи. Об'єктом дослідження був штам *P. multocida* «Смол». У роботі визначали та порівнювали культурально-морфологічні, біохімічні властивості, вірулентність (LD₁₀₀) та резистентність до антибактеріальних засобів штаму *P. multocida* «Смол» в S та R формі. При вивченні культурально-морфологічних властивостей штамів визначали характер росту на рідких (МПБ) та щільних (МПА) поживних середовищах. Біохімічні властивості штамів вивчали на середовищах Гіса з цукрами[3]. Вірулентність штамів для білих мишей (16-18 гр.) визначали методом титрування і визначення величини LD₁₀₀, в розведеннях від 1 до 10 ступенів. Розрахунок величини LD₁₀₀ проводили за методом Кербера в модифікації Ашмаріна [4] в програмі Excel. Антибіотикорезистентність штамів визначали стандартним дискодифузійним методом. У роботі використовували комерційні диски антибактеріальними препаратами. Математичну обробку результатів досліджень виконували за допомогою методів варіаційної статистики за Лакіним Г.Ф. (1980).

Результати та обговорення. На щільних середовищах 24-годинна культура *P. multocida* «Смол» в S-формі утворювала однорідні колонії діаметром 2-4 мм (табл. 1); в R-формі – однорідні колонії діаметром 4-6 мм.

Таблиця 1 – Культурально-морфологічні, біохімічні та патогенні властивості штаму *P. multocida* «Смол» (M ± m, n = 5)

Показники	Штам <i>Pasteurella multocida</i> «Смол»	
	S-форма	R-форма
Біохімічні властивості		
Глюкоза	+++	++
Сорбіт	++++	++
Маніт	++++	++
Цукроза	++++	++
Ксилоза	-	-
Маноза	-	-
Вірулентність		
LD ₁₀₀	LD ₁₀₀ - 0,5x10 ¹	-

Примітка: + - відповідає розчепленню 25%, ++ - 50%, +++ - 75%, ++++ - 100% середовища Гіса після 24-48 годин культивування штаму; +- затримка реакції (96 год); - - негативний результат