

ABSTRACT

Romania pays special attention to development of livestock sector growth through the introduction of sheep and goat breeds with high milk production and genetic improvement of indigenous characters. For this purpose there were created optimal conditions for maintenance and proper nutrition, and the genetic potential of animals is scientifically directed towards high yields of milk and meat. Indeed, sheep and goat, are considered as the most profitable animals by economists and live stock breeders. These, would greatly increase profitability if the morbidity and mortality due to breast disease were reduced. Therefore, the farmer must know the consequences of improper milking for both animal and mammary gland health and the consumer of milk, dairy products and general economy. The main consequence of an incorrectly milking is mastitis. These are inflammation affecting the secretory epithelium, the lining of milk ducts and sinus galactofor or interstitial tissue. Can be caused by mechanical, thermal, chemical, food, and biological (bacteria, viruses, fungi) in the latter category, mastitis “specific” are produced by high pathogenic bacteria (*Staphylococcus spp.*, *Bacillus spp. coli*, *Mycoplasma spp.*, *Listeria spp.*, etc).

Localized inflammation in the mammary gland are more common in specialized animals for milk production and are produced by a diverse microbial flora, and milk quality is ensured by the mammary gland health, wich produces it primarily and also by processing conditions and recovery. There are multiple sources of contamination and they occur from infected mammary gland and continuing during harvesting, handling and processing of milk. Thus, when practicing milking, the farmer can be the main source of contamination. Bacteria from the mammary gland or added during the harvesting and processing of milk, develops when the temperature exceeds the limit of conservation (4°C) during transport or containers from farms and factories. Besides, milk is an excellent medium for the cultivation of many bacterial species. Milk from these animals has significant physical, chemical and bacteriological which are felt in the process of preparation of dairy products.

The importance of breast disease is particulary so in terms of economic and hygienic milk production by impairment in the affected compartments. Thus, the economic importance of mastitis

is due to: -decrease milk production; -the actual removal of sheep with breast disease; -improper removal of milk or dairy consumption; -high cost of treatment of sheep with clinical mastitis; -the cost of preventive treatment sheep in the dry period and biological products to prevent breast infections; -the cost for the laboratory to diagnose mastitis; -the cost for veterinary care.

It also presents important breast diseases care, the possibility of transmission by milk and dairy products of some very serious diseases being well known. Thus, *Staphylococcus aureus*, for example, easily multiply in milk and produce a thermostable enterotoxin, extremely dangerous for the consumer.

Since the area of activity of CSV Smardan, Galati, there are reported numerous cases of mammary gland diseases, we aimed , on the one hand, to perform a comprehensive study of bacterial mastitis and secondly to highlight and analysis of risk factors involved in the emergence and evolution of bacterial mastitis in sheep and goats.

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The paper includes 130 pages, being written in 11 chapters and is structured according to the rules in force, in two parts. The first part (chapters I, II, III), comprising 39 pages, representing 30%, summarizes the main bibliographic database of literature regarding bacterial mastitis of sheep and goats and is the „Current State of Knowledge”.

Part 2 is extended on 91 pages, which is 70%, comprising chapters IV, V, VI, VII, VIII and IX and refers to results of investigation etiology, epidemiology, clinical and immunological taken and considered „researches”. Each chapter in Part 2 has included material and methods, results and conclusions of their discussions part.

In Chapter X, entitled ”**Conclusions and Recommendations**”, are summarized in the 20 main issues final conclusions drawn from the investigations. Based on data provided by the results, for the supervision and control of infectious bacterial mastitis in sheep and goats on the jurisdiction of the CSV Smardan, Galati, there has been made 7 recommendations.

The paper has a total of 77 figures, 22 tables and it’s based on 111 bibliographic titles.

We believe that, on one hand, newly synthesized bibliographic information in the first part of the paper, on the other hand, the results and conclusions from their research, will be a real help to a better understanding of the prevalence of bacterial mastitis sheep and goats and the role of risk factors favoring the emergence and developments.

Chapter I, titled ”**Bibliographic data on bacterial infectious mastitis in sheep**”, plays the main general aspects such as bacterial infectious mastitis in sheep and goats.

Mastitis is defined as inflammation of the mammary gland processes which produce physicochemical and microbiological changes, with a complex etiology. After Beslin and col. (1980) when speaking of the etiology of mastitis must consider two main factors involved, namely: biotic and abiotic factors. Mastitis occurs and evolves as a result of the mutual action of nonspecific factors (abiotic) and the specific (biotic).

Microbial etiology of mastitis in sheep and goats, includes a wide range of bacterial species. Commonly isolated bacterial agents and generally considered culprits of infections and lesions of mammary gland tissue are staphylococci, pasteurella, mycoplasma, coliform bacteria, etc...

Etiological classification criterion of mastitis is most commonly found in the literature and adapted to current practice, giving importance to one or other responsible in some degree of the clinical and pathological breast infection process. In certain cases, are produced mixed bacterial infections (bacteria or yeasts, viruses bacteria rarely) especially in chronic mastitis, but the clinical and pathological stay the same.

The main mastitis bacteria found in sheep and goats are staphylococcal mastitis, pasteurella mastitis and mycoplasma mastitis.

In **Chapter II**, titled "**Bibliographic data on gangrenous mastitis (*Staphylococcus*)**" are currently available data on the knowledge of gangrenous mastitis of sheep and goats. It reviewed the history of gangrenous mastitis knowledge about the importance of economic and cultural morphology of the etiological agent (*Staphylococcus aureus*), epidemiology, pathogenesis, diagnosis, and control prophylaxis entity.

In **Chapter III**, titled "**Bibliographic data on contagious agalactia**" the data on the knowledge of *Mycoplasma agalactiae* infection in sheep and goats. It reviewed the history of knowledge *Mycoplasma agalactiae* infection, the importance of economic and cultural morphology, pathogenicity, epidemiology, pathogenesis, clinical and pathological, diagnosis, prevention and control of disease.

Chapter IV is played "**Purpose and objectives**".

Infectious mastitis bacteria such as inflammation affecting the secretory epithelium, the lining of milk ducts and sinus galactofor or interstitial tissue. These localized inflammatory processes in the mammary gland are more common in specialized animals for milk production and due for a diverse microbial flora.

The presence of bacterial mastitis in sheep and goats caused economic losses due to mortality, crawling animals, necrosis and breast disease, decrease or total or partial disappearance of milk production, as well as treatment costs due to application led us to undertake investigations that followed objectives:

1. Research on the prevalence of bacterial mastitis in sheep and goats.

2. Research on gangrenous mastitis (staph) in sheep and goats.
3. Research on contagious agalactia in sheep and goats.
4. Evaluation of the immune response after vaccination against staphylococcal mastitis.
5. Evaluation of the immune response after vaccination against contagious agalactia

Chapter V, entitled "**Research on the prevalence of bacterial mastitis in sheep and goats**" highlights based on data included in the consultations and treatment and analysis bulletins issued by DSV Galati, that in the period 2008-2012, the area of activity CSV Smardan, Galati, developed 7 episodes of bacterial infectious mastitis, 3 gangrenous mastitis, representing 42.85% and 4 (57.15%) of mastitis agalactia.

Also, the analysis of the same data shows that, the main risk factors in the emergence of infectious mastitis bacterine had sheepfolds location, milking, mammary gland and teat conformation season and uncontrolled movement of animals.

Chapter VI, entitled "**Research on gangrenous mastitis in sheep and goats**", presents the results of research in two episodes occurring during 2009-2012 in sheep and goats from two stan , the joint effect of the range of activity of CSV Smardan, Galati.

Factor favoring the emergence of gangrenous mastitis in goats (2009) was the udder conformation mechanical accident prone, especially since animals grazing in an area with thorns and shrubs, and in sheep (2012), lesions in the mammary gland during milking sheep by hand by squeezing the breast between the two hands of milker.

Pathogen shedding from the healthy sick animal was favored parlor, on the one hand there isolated sick animals, on the other hand they were milked with the healthy and mastitic milk being spilled on the ground obtained contributed to the spreading of the pathogen and the creation of the other sources of infection.

Clinic, of 140 lactating goats, gangrenous mastitis (2009), affected 16 which is a morbidity of 11.4%, of which 7 have died, representing a mortality of 43.77% instead of the 310 lactating sheep (2012), fell ill 16 (5.16%) and died 5 (31.25%).

Gangrenous mastitis, evolved superacute 9 animals (5 goats and 4 sheep), representing 28.12% and acute in 23 (11 goats and 12 sheep), representing 71.88%.

Sowings made on the usual media (agar and nutrient broth), the average hiperclorurate (liquid and solid-Chapman) and the selective Baird-Parker with mammary secretion collected from sheep and goats with clinical signs of gangrenous mastitis, allowed isolation pure culture of *Staphylococcus aureus* etiologic agent identified based on morphological characters, cultural, biochemical and pathogenicity.

Staphylococcus aureus strains isolated from the two foci of gangrenous mastitis (from goat and sheep), fermented glucose, lactose, mammoza, manita, maltose, sucrose and trehalose, xylose

were inactive against and has been shown pathogens in vitro to test - blood hemolysis of sheep and rabbit plasma clotting citrated sheep and rabbits on the Chapman mannitol fermentation and catalase activity.

In **Chapter VII**, entitled "**Research on contagious agalactia**" investigations have shown that illness occurred in April 2009 in a flock of 322 sheep, of which 191 were infected, representing 59.31%. Increased proportion of disease (59.31%) explains the part contagiousness of the disease, expressed by a high effective diffusivity on and off, and on the other hand shows the presence of risk factors that favored the emergence and diffusion of disease.

The clinical examination revealed that of the 191 sheep with symptoms, 34 (23.04%) developed acute disease, and in 157 (76,96%) subacute localized.

The evolution of acute animals showed anorexia and lethargy, hyperthermia (> 41oC), muscle tremors, swollen lymph nodes, conjunctival inflammation and lameness. Evolution lasted 5-7 days and ended by death in 14.41% (5 sheep) of cases.

Subacute evolution, manifested by symptoms depending on the location of the infectious process. The location of the breast, evolved from 139 (88.84%) and lactating females manifested as mastitis, benign or low-grade fever without fever, congestion and sensitivity of one or both breasts. Also were found quantitative and qualitative changes of lactation. Quantitative lactation decreased or ceased, and quality becomes a viscous yellow-green, made from boiled clot. Fresh milk milking and allowed to stand to separate into two layers: one upper and one lower creamy white with coarse sediment.

The joint location (7.64%), animals limping, weight and preferably moved decubitus. Damaged joints were swollen, warm, painful and volatile. Healing, in most cases occurred after 2-7 weeks evolving.

Locating the eyes or keratoconjunctivitis agalactiae, seen in 6 (3.82%) sheep, manifested by lacrimation, photophobia, conjunctival mucosa and subsequent inflammation of the cornea. The cornea was observed the presence of small white opacities that were extended and fully included cornea.

Sowings made from mastitic milk on medium enriched with glucose and normal horse serum with added thallium acetate (500 mg/ml), penicillin G (1000 IU / ml) and amphotericin B (5 mg / ml), incubated at at 37 ° C under an atmosphere of 95% nitrogen and 5% CO₂ to allow isolation and identification of Mycoplasma agalactiae species.

Chapter VIII, entitled "**Research on the immune response after vaccination in gangrenous mastitis (*Staphylococcus*)**" presents the results of investigations carried out on two groups of goats vaccinated group A (n = 10) used as control and group B (n = 10) Vaccine against gangrenous mastitis. Booster Vaccination was carried out 21 days after the first inoculation. From

these animals, blood samples were collected on day 0 (crop I), day 21 (harvesting II), day 47 (harvesting III), day 64 (harvesting IV) and 6 months (harvesting V).

Immune response after vaccination was assessed by determining the relative and absolute values of serum protein fractions, concentrației value of IgG and lysozyme value. To this end we have used the following operations: agarose gel electrophoresis, single radial immunodiffusion assay, plate lysis method, and determining the Biuret method, and the results were averaged.

Dynamic research of serum protein fractions in goats in the group vaccinated and unvaccinated group showed increased concentration of γ -globulins and decreased albumin levels.

The results obtained show that immunization with vaccine gangrenous mastitis in goats induced a statistically highly significant humoral immune response by increasing IgG concentration, total protein and lysozyme. Immunological parameters studied showed a significant increase after booster inoculation of the vaccine.

In **Chapter IX**, entitled "**Research on the immune response after vaccination in contagious agalactia**" are given the results of the investigation of immunological effects after inoculation agalactia vaccine.

The research was conducted on 20 sheep, divided into two groups: - group A vaccinated (n = 10) used as control and group B (n = 10) inoculated subcutaneously with the vaccine against contagious agalactia "Agavac" dose of 1 ml. Booster Vaccination was carried out 21 days after the first inoculation. Since these animals were taken blood samples vacinării day (T0) 21 days before the second injection (T1), 47 days after the first vaccination (T2), 65 days after the first vaccination (T3), 6 months after the first vaccination (T4) and 12 months after the first vaccination (T5).

To assess the immune response and determination of effects techniques were used: agar gel electrophoresis, radial immunodiffusion test simple dosing lysozyme immunoassay test and Biuret method, and the results were statistically analyzed.

Nonspecific immunological parameters studied induced a humoral response by increasing the concentration statistically significantly higher in protein fractions, IgG, total protein and lysozyme after booster vaccination (T2) and remained high semnificativ and the T5 was significant.

Quantification of specific antibodies by ELISA to *Mycoplasma agalactiae* showed high values after the first vaccination (T1), which remained elevated up to the end of the experiment (T4) and slightly lower at (T5).

Following the results obtained it can be concluded that humoral immunological effectors investigation can provide correct evaluation criteria of effectiveness of vaccination against contagious agalactia.

In **Chapter X**, entitled "**Conclusions and Recommendations**", final conclusions are presented in 20 formulations. Based on data provided by the research results, in terms of the

presence and prevalence of bacterial mastitis in sheep and goats in C.S.V. Smardan, Galati county, to supervise and control have made a total of 7 recommendations.