# SUMMARY

This PhD thesis entitled "STUDIES ON SUBCLINICAL MASTITIS AND THEIR INFLUENCE ON COWS MILK YIELD AND QUALITY" has original character due to research conducted both in Romania, in a dairy cattle farm located in the North-East of Romania, and abroad. Study abroad corresponded to qualitative and quantitative analysis of milk parameters, correlated with sanitary conditions existing in 300 studied dairy farms, located in the Walloon part of Belgium.

The originality of the thesis is due to application of diagnostic methods of subclinical mastitis within the herd and laboratory determination of cell populations existing in mastitis milk. Also were analysed risk assessment that could be farms conditions, including the cleanliness and operation parameters of milking machine and their effect on mammary gland in cows.

The data used in the research were processed in the Faculty of Veterinary Medicine Iaşi, in DSVSA Laboratory Iaşi and within the Faculty of Veterinary Medicine of Liege, Belgium.

The thesis comprises of 219 pages and is divided into two parts.

The bibliographic part extends on a number of 32 pages, consisting of two chapters that present data from the literature on functional and morphological structure of the mammary gland, physiology of the mammary gland, mammary gland examination, diagnostic methodology of mammary gland infections and etiology of subclinical mastitis. Also in Part I is presented the natural / organizational area, where research was conducted. In the first part of the thesis are 15 figures and one table.

The second part corresponds to personal research and has a total of 103 pages and is divided into nine chapters, which present materials and methods, results and discussion and general conclusions. Researches are supported by 20 tables and 76 figures.

The bibliography consists of 253 titles from the literature of our country and abroad. Also in the reference list can be found my personal work titles, published in veterinary journals and presented at the Symposiums organized by the Faculty of Veterinary Medicine Iaşi and Faculty of Veterinary Medicine Bucharest.

Because milk is a basic food and also it represents raw material for obtaining other food products, attention to milk quality is very important, starting from the moment of milking. From the hygienic point of view, milk is considered to be the secretion product of the mammary gland of one or more healthy, rested, well fed animals, obtained by hygienic milking, uninterrupted and complete. The chemical components are found in milk in various forms: in solution (lactose, minerals, pigments and water soluble vitamins), colloidal dispersion (protein substances), emulsion (fat, fat-soluble pigments and vitamins).

By the structure and chemical composition, milk, having a high water content, represents a proper medium for the growth of bacterial flora. Dry substance in milk has an average of 12.5%, represented by fat, protein, lactose and minerals. The remaining percentage of 78.5 is water in which are dispersed all other components. (*Pitkala A. et al., 2004; Şindilar E., 2005).*

Goals and scientific research program aimed to formulate a protocol for diagnosis, prevention and therapy of subclinical mastitis in cows. Research has been conducted on the farm in the NE region of our country, and also on dairy farms in southern Belgium. The study material was represented by milk samples collected from cows from these farms. Scientific methods were conducted to diagnose subclinical mastitis in cows, determining the set of analysis, emphasizing prevention methods for subclinical mastitis in cows and determining proper therapy in subclinical mastitis in cows.

Detection of subclinical mastitis is a complex operation, as the appearance of the mammary gland is not modified, milk does not undergo obvious changes and looks apparently normal (*Bradley, A. J. şi col., 2002, Leach, K. A., 2008*) . After analyzing the milk, somatic cells can be detected above the maximum allowable limit imposed by European Union, and also the presence of pathogens can be observed. Meanwhile, milk production decreases, which can coagulates on boiling. Control of subclinical mastitis in dairy cows must be done periodically. For this purpose we used the following methods: California Mastitis Test, R-Mastitest method, electric conductivity of milk, somatic cell counts or counting total number of germs/ml milk.

The large number of somatic cells in milk is a major indicator of chronic or subclinical mastitis. If milk somatic cell count is consistently over 400.000/ml, the ferm is suffering significant economic losses due to the loss of milk and the decreasing the milk price. Since the maximum accepted value is 400.000/ml somatic cell, it is necessary to determine, monitor and

evaluate this parameter by a standard method (*Regulation EC 853/2004 of the European Parliament and the Council of 29.04.2004, EC Regulation no. 854 / 2004 of the European Parliament and the Council of 29.04.2004, Regulation no. 2076/2005).*

Somatic cell count determined by sampling from ferm milk tank must occur frequently in dairy farms, usually twice a month, and this method should be the most representative way to assess the health of the mammary gland (*Reneau, J.K., 2005, Djabri B., 2002, Dohoo R, Van den Borne BHP şi col, 2009, Cristina Bulbaşa (Panaite) 2009*). Interpretation of results from somatic cell count milk sample can be influenced by several factors, such as the actual prevalence of infection, the pathogen agent - the streptococcal mastitis has a greater number of somatic cells than mastitis produced by Staphiloccocus aureus *(Hohmann, K., 2007, Boor KJ, şi col., 2001, Suriyasathaporn, W.,2000),* animal age (prevalence increases with age of the animal), the climate - mastitis are more common in summer, as observed by *Olde Riekerink* (2007) and later by *Bradley, A. J. and col., 2007.*

Using the method of counting somatic cells from farms milk tank is useful for determining the quality of milkthat should be transported to distributors. In the presence of a single cow with high somatic cell number, it is possible that the number of somatic cells from milk tank is not increased, but the presence of more cows with mastitis, the milk tank somatic cells increases significantly (*Hocquette Seegers H., şi col, 2000, Ma Y., 2000, Schukken YH, 2003, Hocquette J.F. şi col., 2005, Wenz J. R., 2007*). Even if the animal is not infected, milk can be contaminated with bacteria during milking, by using the milking machine, all the way to the milk tank. Among pathogen agents, the most common are coliform bacteria (*Escherichia, Enterobacter, Klebsiella),* which ferment lactose to form gas. Due to their presence, somatic cell count increases.

The total number of germs does not match the number of somatic cells, because the parameters have different causes in general. The number of somatic cells increases by the pathogenity of infection of the mammary gland, while the number of germs indicates low hygiene conditiond (*Dürr J.W., 2008*). Currently there are many devices that can measure the total number of bacteria in a sample of milk.

Microbiological examination must be conducted regularly among milk processing units and can be accomplished by: determining the total number of germs (TNG), bacterioscopic

examination of the analyzed sample, the isolation and typing of the etiologic agent, pathogenicity research of the isolated etiologic agent. The total number of germs can reflect the expression of mammary gland health and milk hygiene *(Perianu T., 2003, Cristina Bulbaşa (Panaite), 2010*). In healthy milk at 30 ° C are located bacteria from several thousands to 100.000/ml (*Makovec, JA et al., 2003*). Determine the number of germs in milk is not a sufficient method to diagnose mastitis, as milk may become contaminated even due to errors during cooling milk, during milking because of low hygiene or the milking machines or employees. In these cases increases the total number of bacteria in milk (*Makovec, JA, 2003, Libby S.J., 2004, Bradley A.J., 2002*).

Etiological agents of mastitis can be numerous: bacteria, fungi, rarely viruses, but the more common is bacterial infection, which is the most important. Common pathogens incriminated in the occurrence of subclinical mastitis are: *Staphylococcus aureus*, *Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis* and *Escherichia coli* (*Costello, M, 2003, Wall, R.J., 2005).*

Most authors *(Frost şi col., 1997; Drugociu D., 2001, Mottram şi col., 2002, Hamann, 2005, Schukken, Y.H., şi col, 2008, Viguier Caroline, 2009)* agree that a single test is not enough to diagnose subclinical mastitis and many tests should be combined.

Highlighting the influence of subclinical mastitis on milk quality and production of the cow was also an important objective in developing the work. Since milk is a basic food in human nutrition, its quality should be monitored carefully, even from the farm, during the milking time. Diagnosis of subclinical mastitis if the herd observed quality parameters analyzed samples. Inflammation causes a decrease in mammary glandular synthesis, which lowers the concentration of fat and casein in milk *(Shennan, D.B, 2000, Pyörälä, 2003 şi Akers, 2002).* Also, studies conducted by *Hortet & Seegers (1998*) have shown that subclinical mastitis can produce a decrease of the percentage of fat and in the same time the protein percentage increases. This is due to the presence of the large number of inflammatory proteins, coagulase-negative, while the ratio of casein decreases *(Bruckmaier R.M., 2004; Auldist et al., 1995; Leitner, G. şi col., 2004).*

Subclinical mastitis may produce milk composition change in different proportions, depending on the pathogenicity of causal agent. This significantly alter both the number and

ratio of types of cells that are eliminated through milk, although milk shows no significant physicochemical and organoleptic changes. Changes in mammary gland tissue appear long before clinical signs appear. The observed changes in viscosity of milk, change of milk pH and density, leukocyte infiltration (increase in somatic cells), vascular leakage, resulting in alterations of milk composition by increasing the number of polymorphonuclear cells and the increase of number of microorganisms / ml milk *(Kelly AL. şi col., 2000)*. Neutrophils are present in normal milk (1-11%), macrophages (66-88%), lymphocytes (10-27%) and epithelial cells (0-7%) (Stressa, 2000, Usturoi M.G., 2008). Macrophages cells play an important role during mammary gland infection, initiating the inflammatory response. Polymorphonuclear cells are thus attracted into milk to distroy bacteria *(Marion Boutinaud, 2004, Cotea C., 2004, Carp-Cărare M. şi col., 2002, Turcu, D., 2004).*

Research has shown that all females diagnosed positive with subclinical mastitis had positive response to the pH test, test performed using indicator paper Albrom.

Determination of freezing point did not provide data regarding the correlation of this parameter with the development of subclinical mastitis, as all analyzed samples were within the limits of $-0.55^0$C and $-57^0$C, being considered a media of about $-0.555^0$C.

Females with production of over 25, liters of milk / day showed an average fat content lower (3.12%) than females with lower milk production (3.2%). Comparing the two groups of animals analyzed, the standard deviation was relatively close.

Average of the total protein concentration for both groups is part of the normal limits, being higher for the group of cows with higher milk production (3.24%) than females with lower milk production (3.1%).

Another important objective of this thesis was to correlate the quality and quantity of milk hygiene and operating parameters of the milking machine during milking time. The study was conducted in Belgium and were analyzed the existing milking machines on Belgian dairy farms, linking the health of the mammary gland to the level of hygiene and operating parameters of milking machine. The study was conducted on 315 farms in the south of Belgium, of which 293 (93%) farms were specialized in milk production, and 22 farms (7%) had mixed production.

The research goal for studying milking machine within dairy farms was based on analyzing the components and operating parameters of milking machine, and then to correlate all these parameters with milk quality (relative to the number of somatic cells) and mammary gland health. Many authors have confirmed that milking machines can play an important role in mammary infections, and also milking machines can have little effect on the occurrence of mastitis if they are properly used and operated in accordance with manufacturer's specifications *(Don Calhoun, 2001, Graeme Mein şi col., 2004, Pamela L. Ruegg şi col., 2008).*

Thus, the milking machine may have a traumatic effect on the mammary gland. Improper functioning of the milking machine is the main reason for the change of nipple appearance after milking: color changes, edema, teat canal opening, the duct lesions or papillary hyperkeratosis *(Srairi. T.M., 2005, Theron, Léonard, 2011, Cristina Bulbaşa (Panaite), 2011).*

Traumatic effect of milking machine on the cow's mammary gland can be seen by the appearance of a nipple ring compression, when milking time is extended, or vacuum level exceeds the normal range. Milking time extended can facilitate lesions of the nipple, or peak hyperkeratosis, lesions that can facilitate pathogens to enter into mammary gland tissue.

In the farms that were surveyed, it was observed that the milking machine can also act as a vector and agent of transmission of pathogenic bacteria from one cow to another. This is facilitated within farms where milking is performed on all cows at the same time, regardless of health status *(Rasmussen D.M., 2004, Osterman S. şi col., 2005, Theron, Léonard şi col, 2009).*

Also, the milking machine can cause cross-contamination with pathogens from the same cow from an infected nipple to another healthy one, if there are fluctuations in the level of vacuum. For this reason it is required a frequent detection of the level of the existing vacuum for each milking machine.

Appearance of mammary gland inflammation has negative influence on production parameters and hence the strong economic indicators *(Wheadon MC, 1990, Shuster, D.E., 1991, Morin D. E., 1993, Østerås, O., 2000, McCrory, L., 2001, White, S. L., 2002, Hogeveen H., 2003).* All dairy cows are subjected to risk of mastitis, from first to last lactation (*Davis, C.W., 2005, Dalton, T. J., 2005*). Economic losses due to subclinical mastitis are caused by a significant decrease in quality and milk production, high cost of treatment, degree of transmission from one animal to another, or further reform of the animal *(Faye B., 1995,*

*Beaudeau F., 2000, Mtaallah, B., 2002, Bârţoiu I.A., 2004, Østergaard S., 2005, Hagnestam C., 2007, Heikkila A.M., 2008).*

Economic measures that can be taken in case of mastitis could be preventing the mammary gland inflammation or decreasing the infection rate *(Runceanu L., 2002, Jayarao BM. şi col. 2003; Hogeveen, H., 2005, Siegel J.D., 2007 , Van Asseldonk, 2009).* Early detection of subclinical mastitis is good, on the one hand because of the possibility of implementing a treatment to increase the healing rate *(Hogeveen H., 2001, Deluyker şi col., 2005, Stafford KJ, 2005, Steeneveld, W., 2007)* and the other hand, early detection of disease may lead to the introduction of management strategies required to decrease the negative effects of mastitis *(Ravinderpal Gill şi col, 1990, Huirne, R.B.M. 2003, Østerås, O., 2005, Kreigl, T., 2006).*

Due to economic and social importance of mastitis, numerous studies have been conducted in recent years, observing the ways of transmission of mastitis: horizontal transmission from one cow to another (contagious), or environmental pathogen transmission *(Zadoks et al., 2002. Huijps K., 2007).*

If contagious transmission, transmission rate reflects the number of new females infected by a cow with mastitis, in the unit of time. Studies by *Lam et al.* (*2007*) and *Zadoks et al., (2002*) have reported several cases of mastitis caused by major infectious agents. Regarding the transmissionof mastitis path through the environment, prevalence of infection is often used to reflect the newly infected cases.

Decreasing the amount of milk is dependent on the severity of infection, the pathogen agent, the general health of the animal, or lactating period. Thus, for the primipara, milk quantity losses are more severe when pathogens incriminated are: *Staph. aureus*, *E. coli*, or *Klebsiella spp*. In multiparous females, agents responsible for the biggest losses of milk are: *Streptococcus spp*, *Staph. aureus*, *E. coli*, *Klebsiella spp*, and *A. pyogenes (Sears P.M., 2003).* Multiparous females suffer, however, a greater reduction in milk production than heifers *(Bennedsgaard et al., 2003; Hortet şi col., 1998; Inchaisri C. şi col., 2010*). The literature studies mentioned decreased milk production, which varies depending on farm management, race, level of production, or the analytical method which has been used *(Schepers, J.A., 1991, Yalcin, C. 2000, Jansen, J. şi col, 2010).* The presence of mastitis in the herd is reported along with the detection of lower milk production and this decrease in production is maintained throughout lactation

*(Schrick F.N. şi col., 2001, Roxström A., Strandberg E., 2002, Seegers H., 2002).* The decreased milk production is the most important economic loss that occurs in mastitis (*Huijps et al., 2008; Hortet & Seegers, 1998; Degraves & Fetrow, 1993*).

Changes occurring in milk composition during mastitis can not be neglected in the economic calculation of mastitis *(Seegers et al., 2003, Oliver, S. P, 2003, Jayarao, B. M., şi col., 2004, Rychembusch V., 2005).* The only changes in milk composition, which have economic importance for dairy producers are those that affect the price of milk, such as parameters that form part of the payment.

Research has also pursued the incidence of subclinical mastitis on the studied farm in our country, following various factors. Age of the animal plays an important role, disease incidence increased with age in females. The frequency of subclinical mastitis in heifers is higher than in multiparous females. Also, specialized researchers have shown an increased incidence of subclinical mastitis in early lactation cows *(Sampimon, O.C., şi col, 2009)*. The occurrence of subclinical mastitis in dairy farms is dependent on the degree of hygiene, including hygiene of the employees, of the mammary gland or of the milking system *(Brădăţan Ghe, 2007, Palicica R. et al., 2007 B. Ferguson, 2007).*

In the four years that lasted the research period, it could be noted that by the seasons, the incidence of subclinical mastitis within the tested herd recorded minimum values in winter and spring and maximum values in the months of summer: June and July and during the autumn months: September, October and also in November.

Depending on the production of milk / day there were observed values of subclinical mastitis incidence lower in cows with milk production between 10-20 liters / day, compared with cows with high milk production.

Cows with production of over 31 liters of milk / day have recorded values of subclinical mastitis incidence higher than cows in the first two lots, with a value of subclinical mastitis incidence greater by 25.8% compared to cows with yields of 10-20 liters of milk / day.

In relation to the distribution of the mammary gland quarters, subclinical mastitis incidence is higher by 10.8% to the quarters situated in the rear that those situated in the front, and 10% higher in quarters located on the right to those located on the left. This is due to low

hygiene at the rear quarters, correlated to a high develop of quarters, no pre-milking hygiene, poor hygiene of the animal rest bed.