Animal farming in transition –
the role of animal reproduction:

Mastitis symposium

Proceedings from a symposium at
the St Petersburg State Academy of Veterinary Medicine, Russia
January 10-12, 2007

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Foreword

The Mastitis Symposium arranged at the St Petersburg State Academy of Veterinary Medicine, Russia, on January 10-12 is part of the network programme "Animal farming in transition – the role of animal reproduction". The following academic institutions are included in the network: The St Petersburg State Academy of Veterinary Medicine and the Academy of Management and Agribusiness in St Petersburg, the Belarussian Academy of Agriculture, the Estonian University of Life Sciences, the Latvian University of Agriculture, the Lithuanian Veterinary Academy and finally, the Centre for Reproductive Biology in Uppsala, the Swedish University of Agricultural Sciences (SLU). The cooperation is financially supported by *"Visbyprogrammet*", the Swedish Institute, Stockholm.

This is the first symposium arranged within the newly extended network that initially involved the three Baltic States and Sweden, which has been successfully running since 1999 with the support by the Swedish Institute.

Mastitis was the topic given the highest priority by the network countries when we planned for this symposium. It is beyond doubts the most costly problem for dairy farmers with impact on production, health and wellbeing of the individual animal and the herd, and moreover, on food quality and food safety.

The symposium offers a variety of information in the field of mastitis, including introductory presentations on the physiology of lactation and the pathogenesis of mastitis. Scientific studies on different diagnostic tools and therapeutic regimens will be described, as well as preventive measures against mastitis. Speakers from all sex countries will provide information about the national dairy production and identify specific problems and current research in this field.

With this common scientific focus on mastitis we would like to create a platform for collegial discussions on the problem and invite to future scientific cooperation in the Baltic region.

On behalf of the national programme coordinators, Drs. Triin Hallap in Estonia, Vita Riskeviciene in Lithuania, Vita Antane in Latvia, Renée Båge in Sweden, Nina Fedosova in Russia and Viktor Lavushev in Belarus, we wish you pleasant reading!

Uppsala and St Petersburg in January 2007

Renée Båge, Nina Fedosova, Kirill Plemyashov and Maria Stakheeva (editors)

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Biology of Milking

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More than 2000 years B.C., Egyptian mural paintings depicted the milking procedure. They knew that the process of milking is extremely sensitive and should be carefully managed. To stimulate milk let down, the calf was kept close to the cow, a routine that is still practised in several countries. During the middle ages the routine feeding during milking was described, but it was not until the 20th century that the physiology behind the milk ejection was understood. Milk ejection was described as a neuro-hormonal reflex. Stimulation of nerves in the teats results in release of the pituitary hormone oxytocin from the brain, oxytocin is then transported via the blood to the udder. In the udder oxytocin causes a contraction of the myoepithelial cells surrounding the alveolus. Milk stored in the alveolar part is shifted to the cisternal cavities. Consequently, milk ejection occurs when milk is shifted from alveolar to cisternal compartments. In addition to the release of oxytocin, the hormones prolactin and cortisol are released in the mentioned order during milking. Furthermore, it has been observed in goats that milking activates a vagal reflex, since the goats start to ruminate when the milking machine is attached. The existence of local regulatory mechanisms for milk synthesis, activated by teat stimulation, have been observed in several species. The endocrine responses during milking and existence of local factors in the udder indicate that milking/suckling is not only a question about milk removal.

In dairy cows and buffaloes 60-90 % of the milk is stored in the alveolar compartment between milkings. It is therefore critical that milk ejection occurs immediately when the milking unit is attached to the udder to avoid bi-modal milk flow curves during milking. Usually it takes 30 – 60 seconds from start of pre-stimulation until milk ejection occurs. How long the pre-stimulation should be depends on the degree of udder fill. In late lactation and with short milking intervals, the cows need more pre-stimulation than cows in early lactation and/or after longer milking intervals. For an optimal udder emptying, the oxytocin levels must be elevated during the entire milking. Several experiments have been done to determine the optimal milking routine, and it has been observed that strict milking routines elevate the lactation milk production.

Some experiments have claimed that it is necessary to reach a threshold level of oxytocin in the blood for full milk ejection and that levels above threshold have no effect on udder emptying. However, it has been observed that teat stimulation performed either during hand milking or by calf suckling, the circulating levels of the hormone are significantly higher compared to machine milking. If the cows are fed during milking the oxytocin concentrations also are significantly higher compared to milking without simultaneous feeding. Feeding during milking has been reported to decrease the residual milk yield, shorten the milking time, increase the milk flow and in some cases increase milk yield, indicating that the threshold theory is not the whole true.

In the past years, research on oxytocin has demonstrated that there are several effects of oxytocin beyond milk removal. Rat experiments have shown that the hormone is involved in maternal behaviour and metabolism, and has growth-promoting effects in the young. In dairy cows, it has been indicated that milking management practices that increase the oxytocin levels affect cow behaviour. When oxytocin levels are high, the cows appear to perform more social interactions, seem to be calmer and are lying down and ruminating more. In the situations when oxytocin are high, the levels of cortisol are lowered, which indicates a physiological pattern of an anti-stress status. That suckling/milking is activating a vagal reflex where oxytocin might be involved has been demonstrated in different experiments. Vagotomy in rats block the suckling-induced release of oxytocin and feed deprivation (simulating vagotomy) decreases both basal and milking-related release of oxytocin in dairy cows. It seems that oxytocin responses require nutrient availability, which makes sense from an evolutionary perspective.

The hormones prolactin and cortisol are two other hormones released during milking. In monogastric animals the role of prolactin during lactation is to maintain lactation, a phenomenon that has been debated for ruminants, but nowadays it is accepted that prolactin might have a function during the established lactation in ruminants as well. Prolactin levels can be influenced by the teat stimulation and lately it has been indicated that prolactin might be synthesised locally in the udder. The effect of the milking related release of cortisol is not fully understood. As mentioned above, it might be involved in the regulation of anti stress. Effects on metabolism have been suggested. In addition, cortisol might be involved in the integrity of tight junctions in the mammary gland.

Mastitis – How Does It Develop and Why Does It Occur?

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Mastitis, inflammation of the mammary gland, is the *response* of the body to invading foreign agents, e.g. an infection, to defend itself. Thus, the inflammatory reaction, per se, is something good but may, unfortunately, also cause damage to the udder tissue. Infection is the most common direct cause to more pronounced mastitis reactions. However, also other foreign substances, e.g. toxins, can cause inflammation, and molecules from minor tissue damage caused by e.g. milking equipment may also provoke inflammatory reactions of a moderate degree. Mastitis results in increased somatic cell count (SCC) in milk. The SCC in normal milk is < 100 000/ml and consists mainly of macrophages (crucial in the recognition of invading foreign agents), neutrophils (up to approximately 15 %) and a few percentages of lymphocytes.

The inflammatory reaction

The appearance of microbes or challenging agents in the teat is provoking a series of reactions in the body. The alarm is initiated by the recognition of the enemy by macrophages, which start releasing inflammatory mediators (e.g. Il-1, Il-6, TNF-α), functioning as an alarm to which various cells in the body react with an inflammatory response, including vascular reactions, fever and liver responses. The released inflammatory mediators activate other immune competent cells and agents, mediating the inflammatory reaction further. The most important in the defence against mastitis is the attraction (by e.g. Il-8) and function of neutrophils, which rapidly arrive to phagocytose and kill the microbes. This is the event that counts for most of the increase in SCC (up to hundreds of millions) in mastitic milk, which is used for diagnosis of mastitis, although also other types of leukocytes than neutrophils are attracted in increased numbers. Other essential changes during mastitis, used in diagnostics, are leakage of blood proteins into milk, altered ion composition of the milk resulting in increased conductivity and pH, release of intracellular enzymes like NAG-ase, and depressed function of the milk synthesizing cells reducing the milk yield and the production of casein, fat and lactose. The enhanced release of various kinds of enzymes e.g. during phagocytosis may cause damage to the tissue and maintain or further speed up the inflammatory reaction, resulting in a prolonged process and delayed recovery. It may also result in a maintained low grade (subclinical) reaction that may go on during considerable time (chronic mastitis). The healing depends on the host-microbe balance; the attributes of the microbe and the efficiency of the cows immune system.

Defence mechanisms of the udder

Before the inflammatory reaction develops there are several lines of defence in the udder, to neutralize or get rid of the foreign agents or microbes.

- The **teat canal** is an efficient mechanical barrier if properly closed. In addition, the mucosa is covered with a keratin layer with antimicrobial properties. To keep the teat canal intact is of outmost importance to prevent infectious mastitis.
- Microbes are washed out by the **milk flow and epithelial desquamation**. Microbes must adhere to the epithelium and start multiplying, before an infection is established. The presence of microbes in milk is not equivalent with an infection. This is important to consider when interpreting results from bacteriological examination of milk.
- In the milk, the microbes are exposed to **antibacterial factors** like lactoperoxidase, lysozyme, lactoferrin, complement etc., always present in the milk in various concentrations, ready to start combating the invaders. The efficiency is, unfortunately, rather poor and some factors have an effect only in cooperation with other agents.
- **Phagocytosis,** primarily by neutrophils, is considered the most important mechanism in the local defence against mastitis. The neutrophil acts rapidly but is incapable of sustained effort. The macrophage arrives later and acts more slowly but is capable of more sustainable phagocytosis.
- During inflammation the concentrations of **immunoglobulins** (Igs; antibodies) increase. Igs and complement enhance the phacocytosis by **opsonising** the bacteria, thereby enabling binding to the surface receptors of the phagocytes, which facilitates the engulfment. Igs also neutralize toxins and may also be directly bactericidal. The main function of the secretory IgA is to cover the surface of the microbes to prevent them from adhering to the epithelium.

Etiology to mastitis

The etiology of mastitis is indeed multifactorial. Even if bacteria are the most common direct cause, many factors can make it more or less difficult for them to invade the udder and establish an infection. The occurrence of mastitis is influenced by 3 different ”systems”: The cow, the environment and the management. Besides the genetics of the cow, any factor that contributes to damage of the tissue in udder and teats, to increasing the infectious pressure around the udder and to impairing the immune defence of the cow is predisposing for mastitis. Several predisposing factors acting in concert are usually necessary to enable the establishment of an individual mastitis case or a mastitis herd problem.

Mastitis Treatment in Swedish Milk Production

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Purpose

The goal was to reveal any trends in strategies for mastitis treatment in Sweden for the last decades. If such trends exist - do they correspond to alterations in bacterial spectra and resistance patterns? Another question was if Swedish antibiotic policy seems to be sustainable. Further an evaluation of existing data bases validity for mastitis incidence was made. Finally the author tries to determine if organised preventive mastitis control is more effective than antibiotics for long time improvement of the udder health on a herd level.

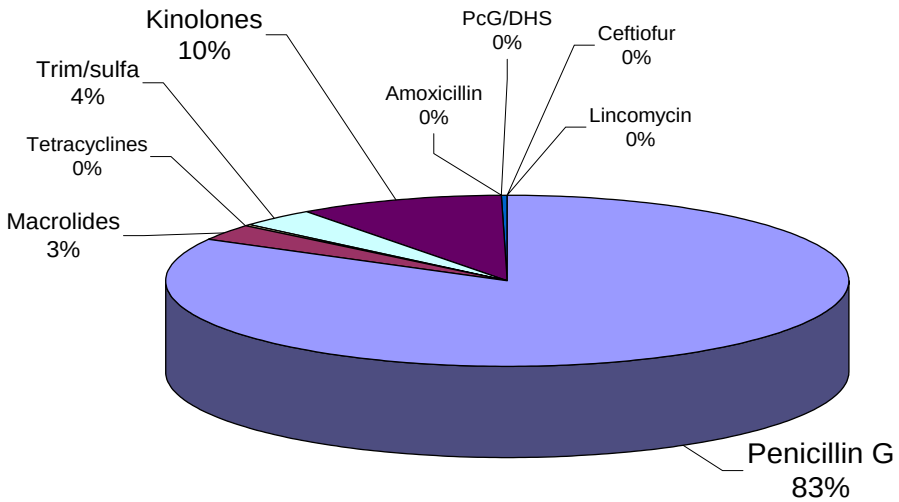
Methods

- Three different scientific studies of representative mastitis cases in Sweden were compared (Funke, 1983; Nilsson et al, 1995 and Bengtsson et al, 2003).
- All field veterinary treatments in Sweden are registered in a central database at the *Swedish Board of Agriculture (SJV)*. Data has been studied for the years 2001-2003. All mastitis cases with the use of any injection antibiotic or local pirlimycin therapy were collected. Regional differences were referred both to region and regional number of milking cows. Also the use of NSAID was evaluated. Different trends in drug of choice in therapy were analysed with a simple Chi2 analyse.
- The Swedish Dairy Association has since early 2000 offered a course; "Management for Healthier Cows", attended since start by more than 500 farmers. Within this course every farms actual *mastitis treatment incidence (MBI)* is registered. Data from 123 farms that have fulfilled the course were compared with the MBI found at SJV.
- A pilot study on 5 high producing Swedish milk herds attending systematic preventive mastitis control (*FRISKKO Udder)* was performed. All farms had a marked decrease in MBI from year 1 to 2 in the program. Short time effect was evaluated as difference in MBI and biological cost (enl. Emanuelsson & Sandgren, 1996) between first two years in program. Long time effect was evaluated by BSCC, percent cows culled for udder disease and percent chronically infected cows in the herd during coming years.

Summary and conclusion

The veterinarian has an important responsibility for antibiotics both in the ecosystem as well as in the human food chain. Inappropriate use can cause problems with resistance among bacteria affecting both humans and animals. Prudent use of all antibiotics is conclusively an important veterinary task not to be neglected. If treatment despite other efforts becomes necessary preferably narrow spectrum substances should be used. Bovine mastitis is the most frequent diagnosis for antibiotic injection therapy in Sweden. Bacterial species and resistance pattern for bovine mastitis has remained unaltered during the last 2 decades in the country. This indicates that the principles of mastitis therapy in Sweden meet the demands of a responsible use. The major guidelines of Swedish mastitis control include restrictive injection therapy with narrow spectrum antibiotics, preferably penicillin G (> 80% of mastitis cases, see figure). The treatment-incidence tends to be correctly registered in different databases, reaching an average level of 23-25%. A pilot study on 5 farms where the udder health program FRISKKO Udder was used for 2 years or more showed a decreased treatment incidence; fewer chronically infected cows, improved milk quality, decreased culling rate and strongly improved economic results.

Drug choice for mastitis therapy in Sweden 2003
Source: Vet@webb SJV – 89.000 registered injection treatments



FRISKKO Udder

The Swedish Concept for Systematic Preventive Mastitis Control

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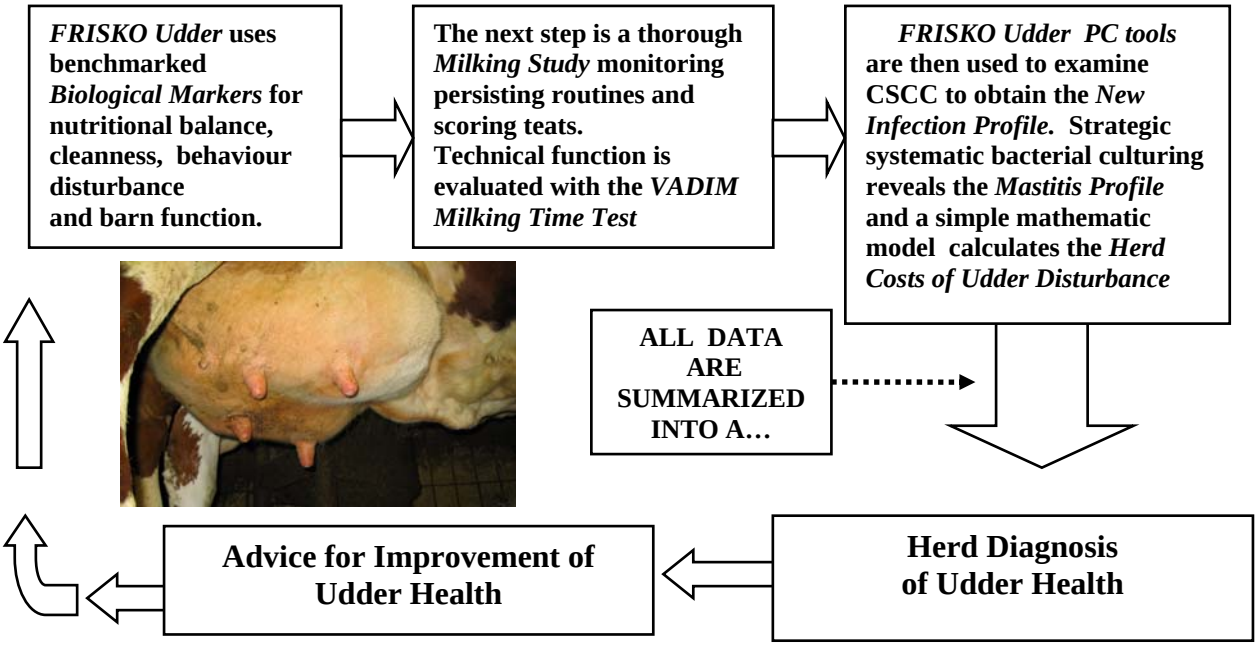
Background

Mastitis is still the most common and costly disease of dairy cows in industrialised countries. Despite various efforts of prevention the single most common action taken by veterinary practitioners or farmers is still treatment of clinical symptoms with antibiotics. In Sweden bovine mastitis is regarded as a multi-factorial production disease with contagious elements. The multi-factorial character and strong association with quality of management creates a need for gathering of data, analysis of herd based risk factors and communication with the farmer in order to prevent mastitis. *FRISKKO Udder* is an attempt to do such work systematically and persistently in order to improve animal welfare, production and profitability in dairy herds.

General methodology

In order to establish profound knowledge about the *Heard Health Pattern* the process starts off in *FRISKKO Basic Service*. When this analysis points out the udder health to be the most important economic and health inadequacy the preventive work proceeds further to *FRISKKO Udder* performed during 2-3 farm visits.

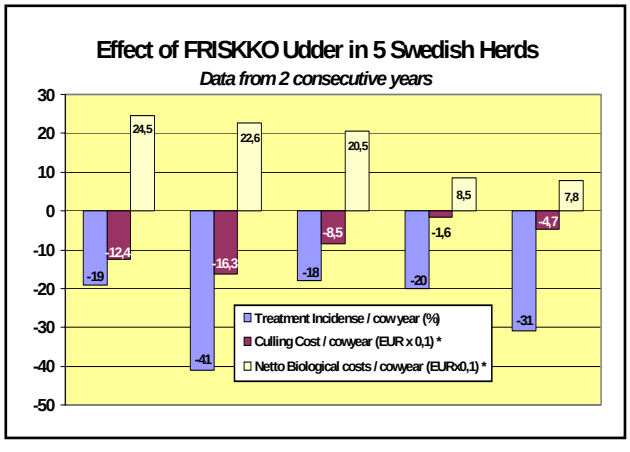


Advice to the farmer

All data gathered forms the *Herd Diagnosis of Udder Health*. Using listed *FRISKKO Risk Factors For Mastitis* the stepwise elimination of management hazards then take place. All *Advice For Improvement* origins from the analysis. Further on the preventive work undergoes *Evaluation* by reactivation of the process.

Results and benefits of the program

A pilot study on 5 farms where the health program *FRISKKO Udder* was used for 2 consecutive years showed a decreased treatment incidence, fewer chronically infected cows, improved milk quality, decreased culling rate and strongly improved economic results. (se graph)



Conclusion: By using the FRISKKO model of preventive work shown above, participating farmers can decrease rates of culling, new infections and antibiotics thus improving both udder health and profitability.

The Analysis of the Etiology, Diagnostics and Treatment of Mastitis in Breeding Farms of Northwest Region of the Russian Federation

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According to the Russian dairy union, the total yield of milk in 2005 in Russia was 31 mil. tons. 40% of this number was converted for milk and for dairy products by 1.7 thousand industries. The middle consumption of pure milk was 245 kl. per one person in 2005. The quantity of milk consumption is rather high, but unfortunately, it is hard to make its quality on the same level. A large amount of new affinities of prophylaxis and treatment and methods of diagnoses appear in modern agriculture. However, such old disease as mastitis remains to stay actual in our days. Mastitis can appear in every farm, in every period of lactation, during every season, mostly in a weak after calving, during the dry period and before the Insemination.

There are different factors that combine grate harm to the agricultural economic system. These factors are:

1. The reduction of quality and quantity of the receiving milk (depending on degree and the type of inflammation).
2. Maternal expenses on the diagnoses, prophylaxis and treatment.
3. Impossibility of delivery production in trade, because of a rest amount of medicine in milk and of the admixture of milk from sick cows (if the general amount of milk contents 10-15% of milk from sick cows, it can not be used for the production of cheeses and sour-milk products).

In some cows, the long lasting inflammation can course the irreversible changes in tissues of dairy iron. It can lead to the atrophy of the struck part of udder and the former yields of milk can not be restored in this case. Over 20% of such cows have to be rejected because of the atrophy of one or several quarters of udder. About 70% of cows from the farm can be stroked by mastitis. Summarizing, all the above stated circumstances, it becomes clear that the mastitis brings a grate economical damage.

Etiology, diagnostics, treatment and the preventive methods of mastitis

According to the importance of the problem, we pursue the following purposes:

1. To study the reasons, coursing the diseases of the dairy iron in cows at the farms.
2. To fined the connection between the mastitis and the gynecological diseases.
3. To determine the role of the microbic factor in the occurrence of mastitis.
4. To define the type of microflora, which courses mastitis.
5. To fined the more affective antibiotics and their duration of action.

The etiological factors, which influence the occurrence of mastitis, are:

- Traumas of udder: bruises, wounds.
- Intoxications (poisonings, diseases of the gastro enteric path, endometritises).
- Infringements of rules of milking (non-observance of rules of preparation of udder for milking, wrong preparation and operation of the milking equipment, insufficient observance of personal hygiene of attendants).
- Infringements of rules of Insemination.

The special value in the mastitis occurrence is the microbic factor, especially in those farms, where many gynecological diseases (25 -27 %) take place.

Interrelation of diseases of sexual system with inflammatory processes in dairy iron specifies the data of the table 1, received at gynecologic inspection of 100 cows in a breeding farm.

Mastitis in cows with gynecologic diseases

The disease	Number of cows	Cows with mastitis					
		The clinical mastitis		The latent mastitis		In general	
		cows	%	cows	%	cows	%
The detention of the placenta	25	2	8	4	16	6	24
Acute endometritis	20	1	5	6	30	7	35
Chronic endometritis	20	-	-	3	15	3	15
Subinvolution of the uterus	20	1	5	2	10	3	15
Abortions	10	-	-	2	20	2	20
Parametritis	5	-	-	1	20	1	20
In general	100	4	5	18	18	22	22

More frequently, the latent mastitis (30%) is observed at the cows with the acute endometritis. The occurrence of mastitis at the cows with gynecological diseases, coursed by microbes, can be explained by the influence of the microbe's toxins on the tissue of the dairy iron. Gynecological diseases can not only course the mastitis, but also to reduce dairy efficiency and to worsen a sanitary condition of milk.

By results of the researches of breeding farms of Leningrad region, we have found out that the most common types of mastitis are: serous, serous-cataralis, cataralis. Such kinds of mastitis as fibrous, purulent and hemorrhagic, occurs rather seldom, in single instances.

We used the common methods, diagnosing the clinical mastitis, according to the general condition of the animal, changes in the dairy efficiency, kind of the excaudate, allocated from the dairy iron.

Researching the milk gathered from ill cows of breeding farms of Leningrad region, it was found out that there are about 200 – 500 somatic cells in 1 ml. of such milk.

The diagnosis of the latent mastitis is also very important. It is carried out once a month. Subclinical mastitis occurs at 40 – 60% of the herd. The diagnosis of mastitis became much easer with occurrence of "mastidin". This method is notable for simplicity of performance and exactness: 1 ml of milk mix with 1 ml. of 2% solution of "mastidin". If the solution is colored with lilac, it specifies absence of mastitis. If there is a clot of violet color in the solution, it means presence of mastitis.

The chemical changers in milk from the cows with latent mastitis are: increase in the contents of chlorides, nitrogenous substances solubled in whey, reduction of level of skim dry rest, lipids, dairy sugar, casein, calcium, calium and lisacinum. Duly detection of subclinical mastitis is very important for the opportunity of early treatment, prevention of development of clinical mastitis. It also lowers the financial expenses. Because of the affection of even one share of udder, the lost of milk for one lactation, can reach 10 -15 %.

In the breeding farms of Leningrad region, the preventive actions are regularly carried out for the lowering of possible occurrence of disease.

The highest therapeutic effect (82%) at mastitis is reached by the duly treatment at the time, when the first sins of the disease appear. It prevents the extension of struck tissue, transition of the acute process to the chronicle, transmission of the infection between the animals.

It is necessary to define the sensitivity of the pathogenic organisms to the antibiotics, because all kinds of microbes have special sensitivity to different antibiotics, and they can become resistible to the antibiotic if long lasting application took place. Therefore, it is necessary to examine the sensitivity of the pathogenic factor to the antibiotics once in a quarter.

Wile researching 128 tests of milk (from the cows with the clinical mastitis) with the bacteriological method (crop on nutrient mediums), we defined the growth of such microbic colonies as: Streptococcus uberis, Streptococcus epidermidis, Streptococcus pyogenes, Staphylococcus albus, Staphylococcus aureus, Escherichia coli, Arcanobacterium pyogenes (Actinomyces), Pseudomonas aeruginosa and others. The dominant growth belonged to the Streptococcus epidermidis.

The sensitivity of the defined microflora to the antibiotics was researched by the discs method. Unfortunately, sometimes the antibiotic treatment is unregular, without the definition of the microbic sensitivity to the antibiotics. It leads to the development of the microbic resistance to the drugs. In our case the number of the resistible pathological factors to the antibiotics, which are more often used in the farms, reached 15 – 25 %.

The general treatment consists of the symptomatic: anti-inflammatory ad anti-hypostasis drugs; and of the anti-microbic therapy. The treatment should be combined with the observance of hygiene and antiseptics. Before milking, all the nipples should be treated with the disinfection solution; the milking equipment should be regularly cleaned before

and after milking with the disinfection solution. The work of the milking equipment should be regularly checked. The milk from the ill cows is always utilized. The milking should be done each 2 hours. The attendants should observe rules of personal hygiene and clean hands with the disinfection solutions.

The therapy of the subclinical mastitis should be better done during the dry period with the antibiotics of long action, because the revealing of the largest amount of sick animals falls at the end of the lactation. Such kind of treatment allows to lower material expenses on treatment and prevents losses of milk. “Orbenin DS, EDS” shows the best results of treatment. This is antibiotic with the wide specter of working from the group of synthetic penicillin, steady to the action of penicillinasa. The structure is: clocksacillin in the form of a salt, bensatinic acids. After the last milking, the medicine enters to all quarters of udder (a full syringe to every quarter of udder). This treatment should be done after the ending of each dry period in the herd. The treatment during the dry period prevents the post-natal mastitis in 82 – 85% and stops the inflammation of udder in 65 – 85% of cases.

Conclusion

The prevention of such disease as mastitis is a complex of actions connected not only with the work of a veterinary, but also with a complex of organizational and economic measures.

The general reason, which provokes mastitis in the post-natal period, is the presence of pathologic factors in genitals. The treatment of the animals should be done right after the diagnosis. In the view of prevention, the antibiotics should be entered during the dry period, because the acting of the medicine in udder in this period is longer.

The treatment of the ill cows is more effective when the sensitivity of the microbes, detached from the stroked quarter of an udder, to the antibiotics is checked.

It is very important also to check the herd on the latent mastitis every month.

Comparative Microbiological Analysis of Quality of Unboiled Milk from Healthy Cows and Cows Suffering from Endometritis

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Experiments were carried out in industrial cooperative "Polar Star" on party-coloured cows of 3-5 calving, with the weight of 510-540 kg, and productivity of 8,000-10,000 kg of milk per lactation. The animals were kept in equal conditions with the same ration.

The aim of the work was to compare the quality of milk from healthy cows with the quality of milk from cows suffering from endometritis.

We were to do the following tasks:

1. to find out content of mesofilic aerobic and facultative anaerobic microorganisms (CFU/ml)
2. to define the quality of somatic cells in unboiled milk
3. to define coliform bacteria

The animals were gathered in 3 groups of 10 cows each. The first group is the healthy cows, the second is the cows with latent chronicle endometritis, the third is the cows with acute catarrhal-purulent endometritis. Before milking the udder was washed with warm water, dried with a napkin, first milk was put into separate dish. Samples of milk were put into sterile test-tubes.

Revealing and calculation of mesofilic aerobic and optional anaerobic microorganisms were carried out according to State Standard (ГОСТ) 10444.15-94. Defining of somatic cells was done according to State Standard (ГОСТ) 23453-90 with the help of preparation "Mastoprim". Defining of bacterium of intestinal bacillus was done according to State Standard (ГОСТ) 30518-97\ P 50474-93. Defining of salmonella bacterium was done according to State Standard (ГОСТ) 30519-97\ P 50480-93.

The content of mesofilic aerobic and facultative anaerobic microorganisms (CFU/ml) in the samples of milk from the first group of cows was in average $4{,}3x10^4$ CFU/ml ($5x10^3$-$1x10^5$), in the samples of milk from the second group of cows was about $8{,}1x10^4$ CFU/ml ($3x10^4$-$3\ x10^5$), in the samples of milk from the third group was $1{,}5\ x10^5$ ($7\ x10^4$-$2\ x10^5$). None of the samples contains coliform microorganisms, including salmonella, in 25g of milk. Defining of somatic cells was done with the help of preparation "Mastoprim". In every sample of milk there were not more than $5*10^5$ of somatic cells.

Conclusion:

1. Mesofilic aerobic and facultative anaerobic microorganisms (CFU/ml) in the samples of milk got from sick animals is higher than in the samples from healthy ones.
2. In the samples of milk from both healthy and sick cows there was no admixture of mastitis milk.
3. According to the results of microbiological research and comparison of indexes shown in table 3 we can conclude that milk got from both healthy and sick cows corresponds to the indexes of Food Hygiene Standard 2.3.2.1078-01 and can be concerned as high quality.

Mastitis Pathogenic Agents' Spectrum in Cows' Milk

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Subclinical and clinical udder infections remain essential problem in dairy herds in Latvia. They are present in all Latvian herds and in almost half of the dairy cows. The yearly incidence of clinical mastitis is 6 % of cows, but incidence of subclinical mastitis is up to 30 %. Mastitis causes severe economic losses due to decreased milk production and increased management costs. Mastitis diseased cows' milk may contain pathogenic microorganisms, which is dangerous for milk consumers' health. For example, *Staphylococcus aureus* is a major bovine mastitis agent and primary human pathogen. Other *Staphylococcus* species, as coagulase negative staphylococci, are opportunistic pathogens and can cause infections and intoxications of consumers by milk and milk products. It is important to study mastitis agents' spectrum and its changes to elaborate prophylaxis measures.

The aim of this investigation is to find out predominating bacterial species causing subclinical and clinical mastitis. The total amount of investigated samples was 398. Somatic cell determination and bacteriological examination were carried out at the Laboratory of the Department of Veterinary Medicine in the Research Institute of Biotechnology and Veterinary Medicine "Sigra" of Latvia University of Agriculture.

Plating was done on Blood agar, Baird-Parker agar and MacConkey agar. Different selective media were used. Colonies of staphylococci were examined to coagulase production by the tube coagulase test. Pastorex Staph-Plus (Bio-Rad, France) latex agglutination test was performed for confirmation of *Staphylococcus aureus*.

Microorganisms were identified using gram-positive and gram-negative kits of BBL CRYSTAL Identification System. Statistic analyses were performed using SPSS 11.0 software package.

Gram-positive cocci from the genera *Staphylococcus, Streptococcus, Micrococcus and Aerococcus* were isolated from 95.7 % of subclinically and 83.1 % of clinically diseased cows' udder secretion samples. From gram-positive cocci, the more frequently isolated microorganisms were from the genus *Staphylococcus* both in subclinically and clinically diseased cows' udder secretion samples – in 74.1 % and 48.2 % cases, respectively. The obtained results demonstrate that microorganisms of the genus *Staphylococcus* predominate in subclinically as well as in clinically diseased cows' udder secretion samples. In Latvia, as in other countries, replacement of predominating agents of mastitis from the genus *Streptococcus* to the genus *Staphylococcus* has been observed.

Compared to subclinical mastitis secretion samples, microorganisms from the family *Enterobacteriaceae* (i.e. *Klebsiella pneumonia, Klebsiella oxytoca, Escherichia coli*) were isolated from clinically diseased cows' secretion samples more frequently – in 10 (11.2 %) cases.

Among the species of the genus *Staphylococcus,* coagulase negative staphylococci and coagulase positive *Staphylococcus intermedius* predominated in clinically diseased cows' udder secretion samples, and *Staphylococcus aureus* predominated in subclinically diseased cows' udder secretion samples. The high proportion of isolated coagulase negative staphylococci reveals pathogenicity of these microorganisms and ability to cause subclinical and clinical inflammations of udder.

The average amount of *Staphylococcus aureus* were 11 200 and 7 900 cfu ml^{-1}, but the average amount of coagulase negative staphylococci were 3 300 and 5 400 cfu ml^{-1} in subclinical and clinical mastitis secretion samples, respectively. The average numbers of *Staphylococcus aureus* and coagulase negative staphylococci was not significantly different in subclinical and clinical mastitis secretion samples ($p > 0.05$). Similarly as in cases of *Staphylococcus aureus* isolation, the count of coagulase negative staphylococci in healthy cows' milk in 96.3 % of cases was not higher than 2 000 cfu ml^{-1}, but in subclinically and clinically diseased cows' udder secretion samples the count of coagulase negative staphylococci in 47.4 % and 69.6 % of cases exceeded 2 000 cfu ml^{-1}.

The average somatic cell count (SCC) in subclinical mastitis secretion samples with the number of *Staphylococcus aureus* below 500 cfu ml^{-1} was significantly different ($p < 0.05$) compared to samples with the number of *Staphylococcus aureus* over 500 cfu ml^{-1}.

Immunization of cows using specific antigens is one of the types of mastitis prophylaxis. *Staphylococcus aureus* antigen is developed and experimentally tested at the Research Institute of Biotechnology and Veterinary Medicine "Sigra" of the Latvia University of Agriculture. The results established that the number of cows, the milk of which contains *Staphylococcus aureus,* after vaccination diminished, which proves the positive role of *Staphylococcus aureus* antigen in the prophylaxis of mastitis.

Changes in Immunological Parameters and Lactose in Cows with Increased Somatic Cell Count in Milk

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Introduction. In 2006 the Latvian Agricultural Data Centre (ADC) recordings show that there are 112 thousand dairy cows with average milk yield 5296 kg per year in Latvia. Milk contains on average 4.37% fat, 3.27% protein and 4.97% lactose. The mean somatic cell count (SCC) in milk is 272 thousand per millilitre for all standardised 305-day lactations, including 143 thousand per ml in the first lactation cows. There are several dairy cattle breeds in Latvia. Among 271.6 thousand of dairy cattle pure bred females most widely spread are Latvian Brown (LB) 187.5 thousand (69%), Holstein Black and White 78.3 thousand (29%) und Holstein Red (HR) 3.0 thousand (1%). Swedish Red and White, Danish Red, Angler and Ayrshire together make up only 1 %. Additionally ADC counts beef cattle breeds in total 12.9 thousand of female animals and 37.9 thousand different mixed breed cattle. Statistics on the cattle population structure in Latvia clearly demonstrates that producing milk is the most important activity and source of earnings for Latvian cattle farmers.

Our farmers have a challenge to increase milk quality. Since the 1st of January 2006 the maximum limit for SCC in milk for sale is to 300 thousand per ml. Many farmers should implement strict mastitis prevention measures in their herds. Therefore we have observed an increased interest in more detailed diagnostics of subclinical mastitis, and compatibility of routinely implemented and in practice less known udder health parameters. There are reports in the literature [4,5] about a possible use of lactose concentration as a criterion for assessment of udder functional status; however many researchers consider this indicator as a questionable one [3]. Also, humoral immunity as well as cellular one plays an important role in the body defense of the cow against pathogens and protection of udder health. Different soluble substances – specific immunoglobulins (Ig) and lactoferrin are responsible for humoral immunity. Lactoferrin is an antibacterial factor, which sensitively reacts to mammary gland irritation and inflammatory process.
The aim of this investigation was to evaluate correlation among udder health parameters, namely, somatic cell count, lactose concentration and immunological parameters in milk, such as immunoglobulins and lactoferrin.

Material and methods. The research was carried out in a 75-dairy-cow herd during one year cycle. Herd sample was obtained from 16 milking cows of analogous lactation period. Milk was sampled from each quarter 4 times during the year. Fat, protein, lactose and somatic cell count was estimated in the quarter milk samples by Milkoskan and Somacount analysers. Immunoglobulins IgG, IgA, IgM and lactoferrin concentration was measured by immunefermentaglutination test. The obtained results were summarised and correlation coefficients among all parameters were calculated by software STATA 9.

Results, discussion and conclusions. A highly significant negative correlation between lactose concentration and somatic cell count ($r = -0.73$) was found. This agrees with earlier reports in the literature [1] that explains a decrease in lactose concentration by three mechanisms, first, decreased synthesis of lactose in the diseased mammary gland, second, reabsorbing lactose from milk into blood, third, due to lactose reduction by some bacteria, e.g., enterobacteria. The increased IgG, IgA and IgM concentrations correlate with higher milk protein concentrations ($r = 0.27$; $r = 0.20$; $r=0.25$, respectively). In contrast to that, there is a medium negative correlation between the lactose level and milk protein level ($r = -0.40$). Looking for a causal explanation, facts are found in the literature, that synthesis of lactose is closely connected only to one of milk proteins – lactalbumin. Other milk proteins do not influence the lactose concentration. In case of subclinical mastitis protein concentration remains high, in contrast to significant decrease of lactose concentration. There is a week negative correlation between lactoferrin and IgG, IgA and IgM concentrations ($r = -0.25$; $r = -0.30$; $r= -0.35$, respectively). Probably, this shows evidence that sufficient concentration of specific immunoglobulins facilitates phagocytosis [2] and fast binding of mastititis pathogens, decreasing inflammation and a need for antibacterial factor lactoferrin.

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The Dynamics of Microbial Contamination in Goat's Milk in Association with the Season

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Introduction. The major part of produced milk in the world is that of cows, and it constitutes approximately 85 %, then follows buffalo – 11,3 %, goats – 2,2 % and sheep – 1,5 %. Goats are used for milk production almost all over the world. According to the literature data, there are 502 milj. goats in the world. In Latvia on 1 December 2006 there were 10707 goats registered, that is 2 thousand goats more than the previous year. Latvian farmers keep dairy goats such as Zane and Latvian local breed. Also on larger farms goat milk is used for cheese production.

In recent years there is a tendency of increasing incidence of acute gastrointestinal tract diseases in Latvia. In 2006 compared with 2005, cases of intestinal bacterial infections have increased by 8 %. In order to provide consumers with a harmless, safe and healthy food, the European Union has introduced in its legislation a lot of quality criteria associated with food safety (6).

At present the following parameters are estimated for fresh goat milk: total count of bacteria (a fixed standart is 500,000 cfu/ml, in raw milk), *Salmonella* spp., *Escherichia coli* or intestinal rod-shaped bacteria, *Staphylococcus aureus* presence not permitted. Besides, the somatic cell count (not more than 1 milj./ml), presence of inhibitors (not permitted) and the freezing point (must not be higher than $0{,}520^0$C) are estimated (7).

The aim of this investigation was to estimate the bacterial contamination in goat milk of a certain farm depending on the season.

Material and methods. There are 100 goats of the local breed and Zane breed in the herd. The average milk yield of a goat was 600-700 kg per lactation in the year 2000. Dry matter was ranging from 10,0 to 13,6 %, fat content from 2,9 to 5,6 %, proteins from 3,0 to 3,9 %, lactose from 4,2 to 5,0 %, and somatic cell count was about 10 thousand per mililitre. Goats were kept loose in a suitable barn. Goats were milked 2 times a day by a milking machine in a milking parlour.

Ten goats were included in the research. Bacteriological indices were analysed in the goat milk according to the standard methods, and flushings of the udder surface also were bacteriologically analysed. Examinations were carried out four times a year taking into consideration seasonality. The somatic cell count was estimated by "Somacount 300".

Results, discussion and conclusions. Total count of bacteria has varied due to year season, but amount of CFU have been with accordance with Latvian legislation. The lowest microbial contamination in milk was in spring – 73 000 cfu/ml, the highest was in summer and autumn, 494 000 cfu/ml and 630 000 cfu/ml respectively. That shows evidence that microbial contamination of goat milk is associated with the season. *Staphylococcus* spp. microorganisms were found most in goats milk in summer period – 58 %, as well as microorganisms of *Enterobacteriacea* family and faecal streptococci, in 24 % and 18 % of cases, respectively. The same microorganisms were found in the flushings of the udder surface, that makes include that they have been introduced into milk from the udder surface and indicates to the errors of observing hygiene requirements. As a universally recognised udder health indicator is the somatic cell count (SCC) in milk. The obtained results show that it was lowest in winter – 386 000 cfu/ml, in spring it increased to 470 cfu/ml, its level was high during all the grazing period and reached the maximum in October – 1015 cfu/ml. In all, it should be pointed out that SCC did not exceed the permitted 1 milj. cfu/ml.

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An Overview about Mastitis and Milk Quality Research Done in Estonia during Recent Years

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The main priorities of the Estonian agriculture are milk and grain production. During the recent years farmers and dairy enterprises have done essential investments to increase milk production and quality. Estonian milk products are able to compete in our own market and in European Community (EU) market in future. Average annual milk production has been increased in our dairy herds during last years and is 6509 kg/year (2005). It is still less than the average European dairy production. However, we have already herds, where production of 10000 kg and more have already been achieved. Increasingly, dairy producers and researchers question the economic justification of an increased production for high-producing cows. Several studies have demonstrated that high-producing cows are at increased risk of infectious diseases (Gröhn et al., 1994; Oltenacu and Ekesbo, 1994; Uribe et al., 1995) and decreased reproductive performance (Nebel and McGilliard, 1993; Marti and Funk, 1994; Kask et al., 2003ab). Improvement of milk production consequently increased the incidence of clinical mastitis and metritis (Uribe et al., 1995; Suriyasathaporn et al., 2000).

According to official animal records the reasons for culling cows in Estonian herds are foremost fertility problems (22.5%), especially establishment of new pregnancy during 90 days postpartum (PP) and mastitis (25.4%).
Present project is continuation of previous reseach project "Assessment of postpartum reproductive performance in Estonian high producing dairy herds and development of measures to improve it" and "Mastitis pathogens in Estonian dairy herds and theirs resistance to antibiotic." Investigations what were performed during last three years, showed that uterine involution, uterine bacterial contamination and bacterial elimination time is not a problem in the investigated herds (Kask et al., 2003). The problem is late start of ovarian function PP. It will prolong the days open period and cause the economic loss for the farmers. In the other project was found, that the most prevalent mastitis pathogens in case of clinical mastitis (n=8781) during 2001-2004 were *S.aureus* (29%), *Str.agalactiae* (10%) and *CNS (*12%) in Estonia (Aasmäe, Kalmus et al. 2003). The most prevalent risk factors to mastitis of cows were milking routine, equipment and environmental problems as type and amount of bedding (Aland 2003, Kalmus 2001).

Objectives of the latest study were to document the impact of some management factors on the occurrence of clinical mastitis in primiparous dairy cows and to identify common udder pathogens of clinical mastitis in freshly calved heifers and multiparous cows on the day of calving (Kalmus et al 2006)

A one-year study was conducted during 2004 and 2005 in 11 selected Estonian dairy herds. Data consisted of 68 heifers with clinical mastitis and 995 heifers without clinical mastitis on the day of calving. Multivariable logistic regression with a random herd effect was used to investigate any association between housing system or the time interval from movement of heifers to the calving facility and day of calving on occurrence of clinical mastitis. Milk samples for bacteriological analysis were collected from affected heifers and multiparous cows on the day of calving. Clinical mastitis occurrence in the study population of freshly calved heifers equalled 6.1 %. Housing system was not a significant risk factor for clinical mastitis of freshly calved heifers.

Moving heifers to the cowbarn less than two weeks before calving in tiestall farms increased risk (OR=5.9 p=0.001) for clinical mastitis at parturition. The most frequently isolated udder pathogens among heifers were *Escherichia coli* (22.1%), *Streptococcus uberis* (19.1%) and coagulase-negative staphylococci (8.8%). In comparison, the main pathogen in multiparous cows with clinical mastitis at parturition was *Staphylococcus aureus* (11.2%). Moving heifers to the calving facilities too late in tiestall farms increased risk for clinical mastitis at parturition. The isolated udder pathogens did not differ significantly in tiestall farms compared to freestall farms in heifers, but differences were found between heifers and multiparous cows at parturition.

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Somatic Cell Count as an Indicator of Mastitis

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Somatic cell count in milk is a measure of udder health as well as milk quality. Dilution due to increasing production can contribute to the decrease of SCC. It has been found that 200,000 cells/ml is the most practical threshold to determine the profitability of dairy farms. Somatic cells are also present in milk of healthy cows, and the increase in SCC is a normal cellular defence against udder infections. Most countries do not widely record clinical mastitis incidences. SCC is objectively recorded on a continuous scale, and it has a higher heritability than mastitis incidence.

The year 1979 was the beginning of SCC registering in Estonia and from 1987 these data are measured and registered monthly in a milk recording scheme. After the accession of Estonia to the European Union (EU), the demands on milk quality were in compliance with the proposed EU legal limit of a 400,000 cells/ml. Similar levels are required in New Zealand and Australia, whereas Canada established the requirement of a SCC< 500,000 cells/ml. Milk with SCC > 300,000 cells/ml, used for cheese manufacturing, has been shown to produce a lower yield and quality of cheese. The pasteurized fluid milk with a prepasteurization SCC of 849,000 cells/ml developed flavour defects and has a shorter shelf life than milk with lower SCC.

Estonia faces several problems with SCC affecting milk quality and udder health. In 2005, 26% of cows were culled due to udder diseases, and mean SCC was between 347,000 to 446,000 cells/ml on dairy farms in Estonia. The objective of this study was to estimate heritability of SCC for different breeds and lactations and the impact of birth-year, lactation, calving month, farm and milking operator on SCC.

Milking procedures having an impact on milk somatic cell count and should be designed in harmony with cow physiology. Pre-milking udder preparation lasted from 12 to 50 seconds and consisted of washing, wiping teats and stripping some squirts of milk from each teat. Udders of some cows' were prepared for as little as 12 seconds. The hormone oxytocin actuates the milk letdown mechanism and it is quite essential that udder preparation last longer. The release of oxytocin from the pituitary is essential for the ejection of alveolar milk. Oxytocin is released in response to tactile teat stimulation. The milking machine should be attached shortly after pre-milking teat preparation if milk ejection is evoked. If milking operators devoted less time to udder preparation, then they devoted quite a lot of time to machine stripping. Delay in attaching the milking unit to the cow had also statistically significant impact on SCC. To minimize over-milking, a milking operator should remove a milking machine as soon as milk flow ceases. The maximum over-milking was 93 seconds per cow. A significant relationship was observed between over-milking and SCC as well as between udder preparation and SCC. A milking operator must work only with the number of milking machines she/he is able to monitor throughout the entire milking process. Delay in attaching the milking unit to the cow had also statistically significant impact on SCC. Environmental mastitis and changes in the milking routine were the critical factors most frequently selected as essential for improvements in milk quality.

Heritability of milk SCC. Concerning heredity, the effect of the sire on the SCC of their daughters was analyzed. On the basis of the data of the half-sib sisters the heritability was calculated. The daughters of eight Estonian Red breed bulls and 13 Estonian Holstein breed bulls were included in our trials. The effect of the bull was essential ($P<0.05$). The differences in milk SCC between the Estonian Red and the Estonian Holstein breed were estimated. The average milk somatic cell count of the cows of the Estonian Red breed was 292,000/ml and that of the Estonian Holstein breed was 393,000/ml ($P<0.05$). Variation in milk SCC for healthy cows results in slightly elevated somatic cell count but seldom to extremely high levels. The differences in milk somatic cell count among the daughters of each bull gave a reason to determine the heritability. The milk somatic cell count heritabilities in the first, second and third lactation of the daughters of Estonian Red (0.11; 0.15; 0.18) and Estonian Holstein bulls (0.09; 0.14; 0.16) were quite similar. From these results we can conclude that the heritability of milk somatic cell count increases with lactation. It can be also concluded, that the heritability of milk somatic cell count increased with the age of a cow. It became evident that 9...18 % of the somatic cell count variability was affected by the hereditary factors and 82...91 % by the environment. In addition there was a tendency that the heritability increased for lactation averages of SCC as the number of tests increased. The milk SCC heritability of the first four months was between 0.05...0.08. The above data indicated, that in order to find the milk SCC heritability, it is useful to consider all the control-milking tests of the lactation period. Despite the low SCC heritability, this may be considered as an auxiliary trait by decreasing the milk somatic cell count and increasing the resistance to mastitis.

Dairy Cattle Production in Lithuania

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In Lithuania 221 thousand animal holders keep 924.6 thousand heads of cattle, of which 465.1 thousand are dairy cows. The average herd size is 2.4 cows and in controlled herd – 12 cows. There must be more than 50% of heifers in the herd to maintain normal reproductive and productive performance.. For the present moment, herd structure in Lithuania is not optimal, because on the average 50.3% are cows and only 34.8% of animals are heifers and the longevity of cows is also sub-optimal. On the average cows are producing milk only for 3.7 lactations. In private farms the longevity is 4.0 lactations and in agricultural stocks only 2.8 lactations. According to the official last years data, 7% of all cows were culled, of which 15% due to low productivity and 28% due to udder diseases.

During the last 5 year period the number of controlled cows increased from 25 till 49% of total cow population and for the present moment there are in Lithuania 432555 cows, 215082 cows are enrolled in milk recording system.

There are two main cow breeds in Lithuania; Lithuanian Black and White (67%) and Lithuania Red and White (30%). According to the last control years (2005-2006) data, 158509 Lithuanian Black and White breed cows produced on average 5643 kg milk, and the average milk fat and protein content was 4.31% and 3.35% respectively. During the same period 48199 Lithuanian Red and White breed cows produced 5434 kg milk, and the average milk fat and protein content was 4.52% fat and 3.49%.

There are 73 breeding herds in Lithuania that enroll 15348 cows. The average milk production in 2005-2006 year in these herds was 6653 kg milk, and the average milk fat and protein content was 4.53% and 3.47%. Milk production increased by 225 kg milk, fat content decreased by 0.01% and protein content increase 0.02% in respect with the last (2004-2005) control year.

In the agricultural stocks 43805 cows are kept with average milk production of 5832 kg, and in private herds 171277 cows are kept that produced on the average 5531kg milk. In comparison with the control year 2004-2005, agricultural stocks (39726 cows) produced on the average 5515 kg milk and in private herds (164679 cows) the average milk production was 5366 kg. This means that milk production in the agricultural stocks increased by 317 kg and in private herds by 165 kg per cow. The highest average milk production per controlled cow was reached in private farm (75 cows) - 9698 kg milk, 3.99 % fat and 3.22 % protein. The best agricultural stock (458 cows) reached the highest average milk production - 8315 kg milk, 4.50 % fat and 3.58 % proteins.

Milk recording (productivity control) started in Lithuania in the year 1907. The protein control started in the year 1965. State enterprise “Animal productivity control” is responsible for keeping, organizing, coordinating and performing animal recording control in all herds of animal in Lithuania. The enterprise was established in the year 1998. The procedures for milk recording are stipulated by RL Ministry of Agriculture Regulation No. 39 - 1381, 2001 “Rules of the milk recording for diary cattle”. The regulations include legal norms arising from Directives 87/328/EEC, 86/130/EEC and 94/515/EEC.

“Animal productivity control” has a total 755 personnel. The milk recording is performed by 44 regional leaders of milk recording and 631 recording technicians. Milk recording of dairy cattle has been performed by methods A and C. Method A is performed by record technicians of SE “Animal productivity control”. The condition of recording with method A4 is performing of two milking during the day in the interval of 12 hours. In some herds productivity control is performed using alternative method AT which is based on the milk quantity from one milking alternating mornings and evenings and taking a sample from that milking. Milk recording by method C is organized by individual herd owners and once per three months milking control is performed by record technicians of SE “Animal productivity control”. In any case it must be at least 11 control milking every calendar year. Supervision is controlled by the State animal breeding supervision service under the ministry of agriculture.

State enterprise “Pieno Tyrimai” is a state founded laboratory for raw milk testing, which is equipped with sophisticated analytical instruments. The enterprise is the country’s specialized central laboratory for milk testing, the main aim of its operation is milk recording of controlled livestock as well as composition and quality testing of all bovine milk samples collected in Lithuania. The enterprise’s special attention has been paid to the assurance of testing accuracy. The enterprise has implemented and is continuously improving its quality management system compliant with the international standard EN ISO/IEC 17025, which speeds up problem solving processes, foresees distribution of employee responsibilities and guides towards achievement of the key aim, i.e. flawless operation. Since 12 January 2001 the enterprise has been accredited by the German accrediting service DAP (Deutsches Akkreditierungssystem Prüfwesen GmbH, certificate No. DAP-PL-3393.00), which acknowledged the laboratory’s competence to perform physical-chemical, chemical and microbiological testing of raw milk in accordance with the requirements of the international standards and to perform sampling of raw milk.

Prevention of Subclinical and Clinical Mastitis of Dairy Herds

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Mastitis results from complex interrelationship between the following elements: environment, cow and microorganisms. The disease exists on different degrees in particular herds, and the mentioned degree can be measured by somatic cell count (SCC) value, including elevated or high SCC at the quarter, cow, or bulk tank level. There is possible to distinguish few types of udder infections, which may require special diagnostic procedures and corrective measures. Existing mastitis is controlled by the use of cow side methods and by laboratory identification of the causing microorganism, followed by appropriate treatment or culling. In the case of clinical mastitis we can visually recognize abnormalities in the milk. We can observe high SCC without visible alterations for subclinical mastitis cases. Subclinical mastitis is the most common form of mastitis.

In 2006 there are about 450.000 dairy cows in Lithuania. The average number of milk recorded cows amounted to 215.000. Milk production per cow – 5592 kg, fat – 4.36% (235 kg), milk protein – 3.38% (189 kg).

The main aim of the subclinical and clinical mastitis control is to avoid this disease in state and family farms. Suitable activities covering organization of subclincal mastitis control are needed to prevent the spread of cow mastitis in particular regions. Mastitis control is carried out at the farm level and at cow level.

At the farm level it is very important to identify mastitis problem herds with elevated SCC in the bulk milk samples. Milk in the bulk tank should contain 400.000/ml SCC. If the SCC is higher, this one indicate subclinical mastitis problem. We have noticed that it is very important to monitor environmental factors, feed ration and its content. Especially we recommend to pay attention to milking routines – washing of the udder with running water, drying with a single used paper towel, removing the first milk streams into control cup, dry teat before milking unit connection. To avoid traumatic mastitis it is necessary to evaluate and monitor technical state of the milking system. Milking equipment should be characterized by adequate size, proper functioning and regular cleaning. Subclinical mastitis treats at drying off with commercially prepared dry cow single dose product. If we determined chronic mastitis, cows have to be separated, milked last and if necessary they have to be culled.

At the cow level we follow individual cow records and other production factors and especially udder health. Value of SCC for individual cows should be monitored as routine activity by using indirect (CMT) and direct methods. When CMT score is 3 or more in one quarter, aseptically milk samples from all quarters of the cow are selected and sent to the district veterinary laboratory for the bacteriological examination and antibiotic susceptibility test. According to the data coming from increased CMT scores or SCC of cow or quarter milk it is possible to find out information about type of microorganisms that can be involved. The culture results are very important for an adequate understanding of specific herd problems, as well as making recommendations for therapy and undertaking other important decisions related to individual cows.

To prevent spreading of contagious microorganisms special control measures and activities are used, i.e. teat dipping, restrictive handling and milking of infected cows. Infections are usually transferred from cow to cow during milking via contaminated teat cup liners, milker hands and shared wash sponges or rags.

Results of some investigations showed that approximately 49 % of cows can suffer in the consequence of mastitis. The studies indicated that 42.6 % of cows have subclinical mastitis. Percentage distributions of quarters infected by subclinical mastitis are: two quarters – 42 %, one quarter – 37 %. The main pathogen of subclinical and clinical mastitis is a contagious microorganism – *Staphylococcus aureus* (46 %). The isolated *Staphylococcus aureus* strains were tested to beta lactamase production. The beta lactamase was produced by 30 % of the *Staphylococcus aureus* strains.
Streptococcus agalactiae was isolated in 6.5 % of the cows. Whereas Coagulase Negative *Staphylococcus* (KNS) and *Mycoplasma bovis* were isolated in 4.7 % and 3.1 % of cows, respectively. *Mycoplasma bovis* is transmitted directly by infected treatment solutions, equipment, milker hands, and milking machine. Sanitation measures are important to prevent the indirect transmission of *Mycoplasma bovis*. Cows in all stages of lactation, including dry cows are susceptible to infection, but most infection occurs during milking. Certainly cows can become chronic carriers. Calves can be infected by ingestion of contaminated milk. As a result of our investigations we can tell, that this type of infection can lead to pneumonia problems (about 10 %).

Environmental pathogen – *Escherichia coli* was isolated in 8.1 % of the investigated cows. *Pseudomonas aeruginosa* was isolated in 3.03 %, *Corynebacterium bovis* in 10.8 %, other in 2.7 % of cases. Contaminated samples occurred in 11.7 % and negative samples in 5.13 % of all cases.

Clinical and subclinical mastitis control should be carried out in co-operation with cow herd owner, local veterinary practitioner, veterinary laboratory workers, and scientists.

Current Activities of Mastitis Research in Lithuania

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Mastitis is thought to be the single most costly disease for dairy farmers in Lithuania. It is estimated that up to 70-80% of all losses in dairy production are due to mastitis. Losses/or extra costs taken into consideration are the following: lost milk production 50 to 55%; animal culling 30 to 35%; costs of treatment and extra labor 20 to 25%. Sub-clinical mastitis is most frequently diagnosed due to increased somatic cell counts (SCC) in bulk milk samples, assessed by milkers and State enterprise Pieno Tyrimai, an accredited central laboratory for milk testing in Lithuania. Due to mastitis, milk quality is affected. For this reason, much energy and resources is spend on a mastitis control program that is focused to implement effective measures for prevention and treatment of clinical and sub-clinical mastitis cases. Research activities in Lithuania are centered 1) to study etiology, infective agents and the causes of high somatic cell count in milk, 2) to assess the efficacy of various commercial and homeopathic preparations in prevention and treatment of sub-clinical bovine mastitis, and 3) to study the mechanism of the early immune defense in acute and sub-clinical mastitis.

Milk quality and composition change in relation to increased somatic cell count is studied in cows and dairy goats. According to Klimiene et al, 2005 each year in recorded Lithuanian dairy herds approx. 10% of cows are culled due to udder diseases and 5% due to decreased productivity, i.e. cows which productivity decreased due to mastitis. According to the National veterinary laboratory data, the main agents of bacterial mastitis were *S. aureus* - 59.74%*, S. agalactiae* - 15.1%*, P. aeruginosa* - 0.62% and *Corynebacterium spp*. -0.97%. Furthermore, sub-clinical mastitis was registered in 90.5% and clinical mastitis in 9.5% of cases. The highest risk of mastitis was detected in 2 to 4 year and in 5 to 7 year old cows during the housing period. Klimaite et al, 2003 states that one of the causative agents is yeasts. In their study, based on 689 milk samples, yeasts as pure culture was found in 2.61% of the cases. In 17.9% of cases, mixed bacterial infection and yeasts were isolated.

Antibiotics are frequently used to control mastitis in dairy animals. The presence of antibiotic residues in milk is very problematic due to the withdrawal time after final treatment, which is associated with increased costs. For this reason, the efficacy of various preparations that have no withdrawal time is tested. Klimaite et al, 2004 tested preparation 'Biomast' (Biomast, Poland) on lactating cows having SCC of 1151.2 ± 227.19 $10^3/cm^3$) drying off cows. The preparation was injected sc, laterally to udder lymphnodes on both sides. At 14 d. after injection of preparation the SSC decreased by 76.2% ($p<0.005$), and total bacterial count decreased by 48.5% ($p<0.005$), 21d after injection, the SSC increased, but was by 34.57% (n.s) lower compared to values prior to treatment. Total bacterial count was by 12.5% ($p<0.005$) higher compared to that on day 14. At 60 days after injection, the SCC and total bacterial count unremarkably increased. Cows that were injected with 'Biomast', before drying off, postpartum had decreased SCC by 17.1% ($p<0.005$), and total bacteria count by 78.9% ($p<0.001$). The same group of researchers has recently tested homeopathic preparations for the control of sub-clinical mastitis. 'Traumeel ® S gel' (Heel GmbH, Germany) was injected into affected udder quarters alone. Also a combination of 'Traumeel ® ad us.vet' and 'Phosphor-Homaccord' (Heel GmbH, Germany) were tested after injection IM. Cows were selected having >500 $10^3/cm^3$. Milk samples are obtained for bacteriological testing, general bacterial contamination, SCC, milk composition prior to treatment and on Days 14 and 21. The results indicate that 'Traumeel ® S gel' cured 37.5 % of affected udder quarters, and a combination of two preparations is evem more effective- 'Traumeel ® ad us.vet' and 'Phosphor-Homaccord' – 53.3%.

For the present moment undergoing studies are to assess the efficacy of low level laser therapy on udder health in dairy cows. For this experiment, cows in their second to fifth lactation with analogous milk production (5000–6000 kg) and having SCC over 300000 c/ml. were selected. The udder of the cow was irradiated once a day for one minute during one week. In all milk samples of irradiated cows, SCC had decreased, showing significant differences ($P<0.01$) after 21 days of irradiation. After irradiation the number of a microbial colonies decreased in some cows. The effect of laser irradiation on microbes is more pronounced following 21 days after irradiation. A significant influence of laser irritation on the udders of healthy cows was notestablished. The authors conclude that low level laser therapy positively influences udder health in cows with elevated SCC.

The most recent study that has started in 2006 year and combines resources of two labs, immunology and animal reproduction, aim to study the mechanisms of the early immune defense in acute and sub-clinical mastitis. We focus especially on events and mechanisms during the early phase of the host defense after *S. aureus* and *E. coli* infection. The following parameters are to be assessed: body temperature, milk yield, consistency of the udder secretions, palpational findings of the udder tissue, general condition, quantification of inflammatory cells in the milk and differential blood counts.

Problems of Mastitis in Cows on the Farms of the Republic of Belarus and Ways of their Solution

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Illnesses of mammary gland in cows are widely spread and cause a great economic loss in animal husbandry due to reduction in milk productivity, restriction in productive use of cows, deterioration of milk quality and dairy products. Among these illnesses mastitis is of special interest. Mastitis is one of the most important problems of dairy husbandry in the Republic of Belarus. This disease develops in cows at different physiological periods and manifests itself in the form of a particular clinical-morphological condition of the mammary gland and change of milk quality.

The problem of mastitis in cows exists in all countries of the world with a highly developed dairy husbandry. The level of illness with mastitis in cows on the farms of our Republic is rather high too and on individual farms it may reach 50%. However this question is not paid proper attention to. Till now there is no serious preventive program for mastitis in the Republic.

Owing to this fact there appeared a necessity of carrying out a constantly planned diagnostic prophylactic and therapeutic work with the development of new means and ways for decreasing the rate of mastitis in cows.

In this direction we have been carrying out the work on farms of the Republic of Belarus. The content of somatic cells in milk has been studied and the relation between the number of somatic cells contained in milk and the degree of morbidity for cows has been determined. In the result of researches of dairy herds for mastitis it has been stated that cases of clinical mastitis are registered rather rarely. The majority of cases are in the subclinical form. In cows, with milk containing from 500 000 till 1 000 000 somatic cells, clinical mastitis in 10% and subclinical in 19.1% have been diagnosed. The clinical form of mastitis in cows, with milk containing 1 000 000 and more somatic cells has been registered in 15.2% and subclinical form in 30.6% of animals.

Practice has shown that there are some difficulties in differentiation of subclinical mastitis from irritation of the udder (secretory failure) that doesn't allow to organize and perform preventive and therapeutic measures more effectively. It has become possible to solve this problem with the help of ionometrical method of diagnostics of subclinical mastitis by the content of chlorine ions in milk.

While studying microorganisms, isolated from mammary gland secretion of cows effected with mastitis we have established that the main causative organisms of this disease are pathogenic streptococci and staphylococci and also mycoplasms. These agents were isolated from the udder secretion in 85.7% (streptococcus - 41%, staphylococcus - 37%, microplasms about 70%) of effected cows.

Corynebacteria (7%), escherichiae (16%) were found more rare. Moreover in 28.6% cases the associations of microflora at mastitis with dominating mycoplasmo-staphylococcal and mycoplasmo-streptococcal associations were registered. One should notice that streptococci and mycoplasms were more abundant in the subclinical form of mastitis.

Staphylococci induced more severe effection of the mammary gland generally at catarrhal and fibrinous mastitis. Corynebacteria were found at purulent mastitis and escherechia - at serous and haemorrhagic mastitis.

It is clear from the mentioned above that it is necessary to evaluate milk for the quantity of somatic cells and microbic contamination, as main indicators of the degree of infection of cow udder and to develop on this basis the effective measures of prophylaxis and treatment. It is known that mastitis is a polyetiological illness and mechanical factors are the greatest group of reasons, causing macro and micro injuries of the udder and teats, due to the poor milking equipment, failure of machine milking technology.

In this connection the use of therapeutic prophylactic ointments for the mastitis prevention is of great concern. We have developed the ointment "Vitemol", which contains an active substance betacarotene. As betacarotene refers to the ecologically pure substances this ointment may be used before and after milking. After its application the reduction of chaps on udder teats was noticed as well as a considerable decrease of quantity of somatic and microbic cells in milk, which led to the decrease till 47% sickness rate of mastitis in cows, a rise of milk performance and quality of milk.

The use of ecologically pure therapeutic prophylactic ointments, diagnostics of subclinical mastitis by the ionometrical method for chlorides is one of the perspective solution of the mastitis problem in cows and the improvement of sanitary and biological quality of milk.

Mastitis and Quality of Milk

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Mastitis is observed in all kinds of animals, has universal distribution without dependence from a season of year, is characterized by long current and the adverse forecast. The inflammation of an udder, in a kind of the wide circulation, is actual enough for the cattle-breeding enterprises, and production received at the given pathology, becomes technologically not suitable. The clinical and latent form of mastitis comes to light on the average in 19 up to 25 % of cows. One of the reasons, considerably quantity of received milk (on the average on 10-15 %) and sharply worsening his sanitary quality, mastitis is lowering.

According to the literary data between dairy gland and sexual system there is a close functional and anatomic interrelation. The reason of disease dairy gland is the infection penetrating into its fabrics on blood vessels from the nearest painful center in an organism (mainly from a uterus). Hence, at gynecologic diseases of cows when in a cavity of a uterus accumulates exsudate, containing microorganisms, in dairy to iron with blood from sexual system act not only pathogenic microbes, but also their toxins, causing infringement of function of formation of milk, a lactation and development of inflammatory process in an udder. Therefore disease dairy gland in many cases (up to 45 %) observe after sorts when optimum conditions for distribution of an infection on blood vessels.

Special value in occurrence of a mastitis has the microbic factor, is especial in those facilities where register the big percent of cows with gynecologic diseases (detention placentitis, subinvolution of uterus, sharp and chronic endometritis). The basic etiology a role in occurrence of mastitises in agricultural animals the microflora (staphylococcus and streptococcus). Presence in milk of microscopic mushrooms testifies to wide application of antibiotics in a facilities.

The numerous scientific researches spent on studying of a mastitis at cows, specify not only change of dairy efficiency and organoleptic properties of milk, but also on decrease in his biological value and sanitary quality, that in many respects depends on the form of a mastitis.

At infringement functions dairy gland occurs not only reduction of a yield of milk and allocation to milk of slime, pus and clots of casein, but also his{its} biological value owing to change of the maintenance{contents}, the general{common} fiber and lactose in its{her} secret is broken. Defeats of fabrics of a udder differing on a degree different forms of a mastitis in many respects differ the maintenance{contents} of somatic cells{cages} in milk, For a subclinical mastitis typically increase of their level concerning those parameters at healthy animals practically three times. At cataralis and purulently-cataralis an inflammation the increase in quantity of somatic cells in one milliliter of a secret up to 2-3 thousand is marked.

Milk of the cows, sick to mastitis, is unsuitable for manufacturing good-quality cheeses, kefir and curdled milk. The system of struggle against the mastitis, based on duly revealing and treatment of sick cows, gives high enough therapeutic effect, however it is necessary to carry out sanitary-and-hygienic actions on a regular basis.

The Ionometrical Method for Diagnostics of Subclinical Mastitis in Cows

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The further intensification of dairy husbandry is restrained mainly by different diseases of a mammary gland among which mastitis is the most important one. The inflammation of udder being widely spread is rather actual for livestock farms and the production obtained at this pathology is becoming technologically unsuitable and dangerous for people health.

The researches carried out by us show that the morbidity of mastitis in lactating cows is 45,28%, subclinical mastitis 28,71%, that indicates poor therapeutic prophylactic measures at this pathology. The presence of great number of mastitis preventive means indicates the necessity of the improvement of diagnostics of mastitis, espcially of subclinical form of mastitis.

The main way of diagnostics of subclinical mastitis in veterinary practice is still the calculation of somatic cells in milk with the help of various rapid tests determining mastitis with the outer active substances. But the calculation of changes of cellular elements quantity in milk does not always reliably indicate the presence of latent mastitis as the content of somatic cells increases in colostrum period, at the end of the lactating period, during heat time, with the age of an animal, at leucocytosis. It leads to the faulty identification of animals diseased with mastitis.

While determining the new diagnostic rate interrelated with somatic cells, which indicates only the inflammation of the udder and does not depend on the physiological state of the cow and the lactation period we have carried out the detailed research of the dynamics of cytological and biochemical composition of milk at mastitis.

A statistical working of figures with the account of the correlation factor obtained from the study of morphological and biochemical rates of milk from cows without udder pathology and in different degrees of mastitis has shown the interrelation between the content of somatic cells in milk and chlorides. So in adult animals having 334,4±11,83 thousand/milliliter somatic cells in milk the concentration of chloride ions is 35,6±0,28 mole/liter, that composes the natural background of chlorides in milk of healthy animals. In the secretion of a mammary gland under the medium severity of mastitis (subclinical or catarrhal) the quantity of somatic cells is 965,7±23,41 and 1646,4±56,16 thousand/milliliter, and chlorides are found at the level 70,4±1,37 and 89,9±4,9 mole/liter respectively, that 1,97-2,52 times more then the number of their concentration in milk of healthy cows. In cases of the severe affection of a mammary gland (purulent-catarrhal mastitis) these figures compose 2215,6±23,62 thousand/milliliter and 109,7±1,64 mole/liter, that 3,1 times higher then in milk of animals without pathology.

We have used this regularity as a diagnostic test at the udder inflammation, having adapted the ionometrical method of research for the determination of chlorides in order to diagnose a subclinical mastitis in cows. Experimentaly by the calculation of somatic cells and determining of the concentration of chlorine ions in milk of healthy cows in different lactating periods and under condition of udder inflammation we have determined the relation between chlorides content and somatic cells under the increase of their concentration in milk more then 49,5 mole/liter the quantity of somatic cells increases more then 500 thousand/milliliter.

The research of changes of molal concentration of chlorides depending on the content of somatic cells carried out on 254 effected with mastitis cows and clinically healthy animals in plc “Olgovskoye”, Vitebsk District allowed to approve the 98% diagnostic efficiency of the developed method.

Under result of carried out researches the method of quantitative ionometrical analysis of milk on the content of chlorine ions has been developed for the diagnostics of latent mastitis with the use of ionometre and ionoselective electrode for the identification of chlorides. At the concentration of chlorides in milk more then 49,5 mole/liter the experimental animal is considered to be diseased with mastitis. The interrelation of obtained results relative to the somatic cells is done in accordance with the graduative table.

The use of the developed method in veterinary practice will allow to rise considerably the efficiency of diagnostics of mastitis and to diagnose in doubtful cases more accurately.

The Morpho-Biochemical Composition of Milk at Different Forms of Mastitis in Cows

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At present the mastitis in cows being widely spread is rather actual problem for livestock farms of Belarus. The production obtained at this pathology loses its technological characteristics owing to the changed composition of milk. The inflammation of udder is accompanied by serious morpho-functional and physico-chemical changes in tissues and a secretion under the study of which the majority of authors as a rule don't take into account the form of mastitis or limit themselves by the research of the quantity of somatic cells and active acidity of milk, what from our point of view is not enough for revealing unknown information of pathogenesis, having characteristic peculiarities for each form. That is why the objective of our work was the study of milk composition at different forms of mastitis in cows.

The researches concerning the study of morphological and biochemical changes of milk composition at mastitis have been carried out in plc "Vozrozhdenie", Vitebsk District. Before the experiment in accordance to the results of clinical examination of animals 4 groups of 10 cows in each with a more widespread form of mastitis were formed. The animals were grouped by the principle of paired analogues. The first group was formed from the cows with a subclinical mastitis, the second one with catarrhal and the third one with purulent-catarrhal, in the forth (control) group clinically healthy animals were included. The material for the research was milk and the secretion with a defined active acidity, chlorides content, general protein, lactose, somatic cells taken from the cows with clinical and latent mastitis.

It has been determined that for each form of mastitis the definite changes of morphological, biochemical and immunological data of milk are characteristic. So, pH of milk at udder inflammation deviates from physiological data (6.60±0.03) revealing its alkalinity till 6.84±0.02 at latent mastitis and 7.04±0.05 at catarrhal mastitis. Owing to this fact the peculiarity of purulent-catarrhal mastitis is in becoming of the pH of milk more acid, till 5.29±0.06 as alternative processes are domineering.

The researches have shown that chlorides content in the isolated secretion much depends on the severity of inflammatory processes in udder tissues, as far as the presence of chloride-ion in milk is not the result of biochemical processes of secretion activity of lactocytes, so the increase of their concentration shows the degree of effection of a mammary gland. At subclinical mastitis their content in milk regarding healthy cows is increasing practically twice and contains 70.4±1.37 mole/liter. At catarrhal and purulent-catarrhal mastitis this index is on the level of 89.9±4.9 and 109.67±1.64 mole/liter respectively.

The content of general protein and lactose in its secretion also show the failure of secretory function of a mammary gland. Thus at a latent mastitis the quantity of general protein and lactose relative to the milk of healthy cows are decreasing on 12.5 and 15.5%, at catarrhal mastitis – on 21.6 and 20.3%, and at purulent-catarrhal milk protein increases on 30.27%, that is connected with the decrease of casein and betalactoglobulin content, but with the increase of immunoglobulin and alfalactoalbumin quantity.

Differed on the degree of the effection of udder tissues various forms of mastitis are distinguished from each other much by the content of somatic cells in milk. The increase of their level from the proper level of healthy animals 3 times is characteristic for the subclinical mastitis. At catarrhal and purulent-catarrhal inflammation it is notable the rise of somatic cells in 1 ml of a secretion till 1646.58±56.16 and 2215.61±23.62 thousand. Similar difference in the quantity of cellular elements of milk shows the intensive migration of leucocytes in it and the increase of the speed of apoptosis in alveoli epithelium, cistern and mammary passages. The increase of somatic cells at clinical mastitis compared with their content at latent mastitis points to the direct relation between the inflammatory process in the udder and the quantity of cellular elements in milk.

At the udder inflammation it is notable the characteristic changes of biochemical and cytological data of milk for each form of mastitis. The study of these changes has allowed defining some details in pathogenesis of this disease more exactly.

Complex Spectrum Electromagnetic Waves Influence for Animal Treatment

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Professor Frohlich of Liverpool University proposed that the sick cell radiates acoustic-electrical vibrations in a wide frequency spectrum including millimeter wave range. At first experiments were made on yeast culture. Yeast was radiated with electromagnetic waves (EMW) of 42 GHz frequency. It was found that the speed of the cells' division increased. When the cell was ill, electromagnetic vibrations appeared on the surface of the membrane. Due to the interaction of the millimeter waves external vibration with the membrane surface a process of the albumen structures spatial "extraction" from the cell's depth takes place and this process returns the cell to its natural state. On the other hand new research results appeal by vibration inside of mithochodrial membranes and take advantage of non-heating millimeter wave for process stimulation of albumen synthesis in mithochondria [1].

The use of only non-heating doses of millimeter wave is not always effective for the treatment. This fact can be explained by the Prigogin theory. If only millimeter wave influence on beings, there is trigger effect. Complex spectrum include the low frequency (LF), millimeter wave (MW) and near-infrared frequencies (IF) have not this [1].

Scientific and practical research in the sphere of the EMW therapy began with the treatment of mastitis. The problem concerning mentioned illness is still actual and difficult. For mastitis treatment we worked out special device. The milk gland processing was made in the morning and in the evening for 3 days. The sample of milk was taken before and after processing. Received results were processed with the help of the statistic and correlation analysis. The cows are continued to be observed and clinic examination and milk analysis are taken place.

During the microbiological examination of the cows' milk gland it was found that 17-38 per cent of all the micro-organisms are of spherical form, 3-42 per cent staphylococcus, 58-80 per cent milk-lactic micro-organisms. During the examination of the cows without symptoms, putrefactive micro flora was also found.

After the first processing the composition of the Gram-negative forms of the micro-organisms decreased. The concentration of the micro-organisms was decreasing constantly and on the 3rd day the single Gram-positive forms of the micro-organisms appeared.

That means, that after processing of the cow's milk gland with our devices positively influenced on the microbiological composition of the milk gland and promotes its recovery. On the 4th day it is possible to receive milk of good quality. Same dynamic of microbiological data was obtained after EMW influence at treatment time of endometritis and stomach diseases by cows. Device uses non-heating doses. Researched of preventive phenomena in case diseases. Our experiments confirmed our hypothesis about parametric influence of the EMW on biosystems which means that regeneration processes take place in the cells.

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