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Prevalence and treatment of subclinical mastitis in cows

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Summary

The examinations were carried out at the Center of Experiments and Practical Training and Laboratory of Animal Reproduction of the Lithuanian Veterinary Academy during the period of 1998-2001. Milk composition and quality estimation was conducted at the State Laboratory for Milk Control.

89 cows (214 quarters) with subclinical mastitis were studied. On the basis of the bacteriological study results, it became evident that the majority of subclinical mastitis was provoked by mixed micro-flora (52.85%) and Staphylococcus aureus (20.32%). Mixed micro-flora consisted of: Staphylococcus aureus and Enterobacter spp. – 6.67%, Staphylococcus aureus and coagulase-negative Staphylococcus – 2.86%, Staphylococcus aureus, Streptococcus spp, Enterobacter spp. – 14.13%, Staphylococcus aureus, coagulase-negative staphylococcus and Enterobacter spp. – 16.19%, as well as coagulase-negative staphylococcus and Enterobacter spp. – 11.1%. Other representatives of pathogenic micro-flora isolated were Streptococcus spp. at 13.49% and coagulase-negative staphylococcus, 2.86%. Pathogenic micro-flora was not obtained in 10.48% of the material.

The effectiveness of treatment depends on changes in somatic cell count, lactose, proteins, milk-freezing point and microbiological tests carried on throughout its course. The effectiveness for treatment of the following preparations was: Leo Yellow - 88.71 ± 5.35% and Tetra delta – 79.49 ± 6.47%, Mastiject Fort®— 66.0 ± 6.6% and Divaccon 28 ± 8.98% and Leocillini – 40.0± 8.94%. The effectiveness was the highest when a combination of Leo Yellow and Leocillini was used for treatment of subclinical mastitis and recovery rose to 93.33 ± 6.44%.

Keywords: cow, subclinical mastitis, bacteriology, treatment

Mastitis, or inflammation of the mammary gland, is one of the most complex and costly diseases of the dairy industry. The biggest looses are caused by one of the form subclinical mastitis (6, 8, 12, 16). One of the main indicators are an increase in the somatic cells count (SCC) in milk. The SCC of milk includes epithelium cells (0-7%) and leucocytes (8, 11). Leucocytes are transported into milk gland directly from blood as a response to chemical substances, released by mammary gland during inflammation. Milk leucocytes consist of 50 ± 10% neuthrophils, 36 ± 9% lymphocytes and 14 ± 2% macrophages (5, 11). The main function of neutrophils and macrophages cells is phagocytosis of the cells transported into mammary gland. In healthy cow milk the SCC doesn't reach $100 \times 10^3/cm^3$. A cow is considered to be sick with subclinical mastitis when the SCC in total amount of milk increase to more than $200 \times 10^3/cm^3$ (7, 8, 13) or when in the last portion of milk of the quarter this count is higher than $600 \times 10^3/cm^3$. The greatest part of new udder infections occur during the first three months of lactation and in 80% cases continue during the whole period of lactation (10). These new infections cause the increase of the SCC. Mastitis is rather common for all breeds of cattle. Looses in production reach about 25% during lactation. Milk production isn't restored in many cases. Irreversible changes in tissues are noticed in some cases and then cows are rejected. The decrease of energetical substances (lactose, fat, protein) as well as changes in protein fractions, are observed in sick cows (12, 13). Subclinical mastitis is widespread in our country as well. The investigations have demonstrated that about 42-47% of cows in Lithuania have symptoms of subclinical mastitis (9, 14).

The bacterial pathogens responsible for infection of the mammary gland may be split into two main categories: major and minor pathogens. Most mastitis pathogens isolated from milk samples testing by the CMT were Gram positive cocci. *Staphylococci* constituted 57% of the isolates, of which the predominant cause of bovine mastitis was *Staphylococcus aureus* (40.5%). Other mastitis pathogens isolated include *Streptococ-*

ci (16.5%), *coliforms* (9%) and *Corynebacterium spp.* (5%) (4, 15, 16).

It was demonstrated by the data of investigations in Germany that the main agents of mastitis are *Staphylococcus*. They are very sensitive to antibiotics (Oxacilin, Rifamycin, Cefoperazon, Cefalotin, Enrofloxacin). The best means for treatment of mastitis caused by *Streptococcus* are Ampicilin, Penicillin, and Enrofloxacin for Coliformial mastitis (1, 2, 4). Wawron W., Szczubial M. (17) were isolated from clinical and subclinical forms of mastitis in cows staphylococci, streptococci and *Esherichia coli* strains and their sensitivity to chlorarphenicol, tiamphenicol and rifaximine. Chlorarphenicol was found to have the greatest ability to inhibit the growth of all the microbs group – 100%.

The aim of the work was to investigate the prevalence of subclinical mastitis in cows and to analyze the medicinal curative efficiency of various antibacterial drugs.

Material and methods

The experiments were carried out in the Lithuania Veterinary Academy´s Centre of Experiments and Practical training and the Laboratory of Animal Reproduction during period of 1998-2001. Milk composition and quality estimation was calculated in State Laboratory for Milk Control „Pieno tyrimai". Somatic cells count was estimated by „Fossomatic" instrument (Denmark) and „Somascop MK 2" (Holland). Fat, protein and lactose content in milk was estimated by „Lactoscope 550" (Holland). Total bacteria count in milk was analysed by „Cobra 2024 – Asterija" (France). Freezing point estimation was made by „Astor – 4000 se" (Italy).

For sick cows milk samples were inoculated on McConkey (for Gram-negative bacteria), sheep blood (staphylococci) agars and Edwards medium (streptococci) („Oxoid", England). For *S. aureus* bacteria estimation was used latex kit „Staphytest Plus Test DR 850" („Oxoid", England).

89 cows (214 quarters) with subclinical mastitis were treated by different anti-mastitis preparations. Milk samples were taken from every quarter in end of milking 4 times: before treatment, before second injection, before third injection and in end of witholding time. Milk samples for microbiological analysis were taken from quarters with subclinical mastitis before treatment and in the end of witholding time. Results of treatment were decided by changes in somatic cells count, lactose, proteins and milk freezing point in treatment time.

Treatment was performed using the following preparations: Leo Yellow (penethamate hydroiodide 150 mg, dihydrostreptomycin sulphate 150 mg, framysetin sulphate 50 mg, prednizolone 5 mg; „Leo animal health", Denmark) – 19 cows (35 quarter), Tetra delta (novobiocin sodium 100 mg, neomicin sulphate 150 mg, procaine penicillin 100 mg, dihydrostreptomycin sulphate 125 mg, prednizolone 10 mg; „Upjohn", Belgium) – 17 cows (39 quarters), Mastiject Fort® (oxytetracycline 200 mg, neomycin sulphate 250 mg, bacitracin 2000 IE, prednisolone 10 mg; „Intervet", Holland) – 21 cow (50 quarters), Mastisan A (benzylpenicillin sodium 100 000 IE, norsulphasol sodium 0,35 g; „Sigfarm", Latvia) – 9 cow (20 quarters), Divaccon (Sol. dimetil-3-oxabutil phosphoric acid dimetil ester, Lithuania) – 9 cows (30 quarters), Leocillin (into muscles) (penethamate hydroiodide, „Leo animal health", Denmark) – 5 cows (15 quarters).

Antibacterial drugs were selected according to a antibiogram.

Date was analyzed by various statistical methods. „Bioban" computer program and „Microsoft Excel 97" were used.

Results and discussion

Cows showed mastitis more often during indoor period ($p < 0.001$), than on pasture. Mastitis occured more often in winter then in other seasons: in spring ($p < 0.005$), in summer ($p < 0.01$), in autumn ($p < 0.001$). Highest bacteria counts were observed in winter ($p < 0.025$) and summer ($p < 0.05$) in comparision with autumn.

Mastitis resistance decreases by increasing lactation time. In third lactation, somatic cells count (SCC) in milk increases 2.12 time ($p < 0.005$), and in 7th lactation 2.7 time ($p < 0.001$) in compare with first lactation. By analyzing SCC in cow´s milk in different months we observed that incidence of cows with mastitis in end of lactation increases, especially from 7th month ($p < 0.025$).

Milk quality is characterised by somatics cells count in mililitres (scc/ml) of milk. After researching individual cows which had 400×10^3 scc/cm^3 we estimate that 13.14% udder quarters milk in beginning of milking had more like 301×10^3scc/cm^3 and 15.7% udder quarters milk in end of milking had over 751×10^3 scc/cm^3. After researching cows which had 310--400 $\times 10^3$ scc/cm^3 we estimate that in beginning of milking 50% udder quarters somatic cells was over 301×10^3 scc/cm^3 and in end of milking 50% udder quarters somatic cells was over 751×10^3 scc/cm^3. Best accuracy in estimation somatic cells is reached by separate analysis of cows udder quarters in end of milking. We can diagnose beginning of subclinical mastitis if somatic cells in end of milking are over 600×10^3 scc/cm^3.

After bacteriological examinations the following pathogenic micro-flora were isolated: *Staphylococcus aureus* – 20.32%, *Streptococcus spp.* – 13.49%, coagulase-negative staphylococcus – 2.86%, mixed micro-flora – 52.85%. Pathogenic micro-flora wasn´t obtained in 10.48%. Mixed micro-flora consisted of: *Staphylococcus aureus* and *Enterobacter spp.* – 6.67%, *Staphylococcus aureus* and coagulase-negative staphylococcus – 2.86%, *Staphylococcus aureus*, *Streptococcus spp., Enterobacter spp.* – 14.13%, *Staphylococcus aureus*, coagulase-negative staphylococcus and *Enterobacter spp.* – 16.19%, coagulase-negative staphylococcus and *Enterobacter spp.* – 11.1%.

Treating with „Leo Yellow" (tab. 1) had effect on 88.71% of affected quarters, where somatic cell count

(SCC) decreased ($p < 0.001$). This demonstrates recovery of udder quarters. In cured cases, lactose amounts increased physiological normal values ($p < 0.001$). Total proteins remained stable (tab. 3), and milk freezing point decreased (tab. 4).

Treating with „Tetra-delta" (tab. 1), resulted in a distinct SCC reduction was observed after three applications ($p < 0.001$). Lactose and milk total proteins increased and the milk freezing point decreased (tab. 4). The effectiveness of the treatment was 79.49%.

Following the treatment with „Mastiject-Forte®" (tab. 1), the SCC increased after the initial application but decreased following the third injection ($p < 0.001$). Levels of lactose (tab. 2) increased to normal values ($p < 0.005$). During treatment, milk total proteins and milk freezing points were not significantly affected by the treatment.

Following the treatment with „Mastisan A" (tab. 1), a distinct increase of SCC was observed after the initial injection, but SCC decreased after second and third injections. Levels of lactose (tab. 2) decreased by 0.32%. Milk proteins and milk freezing point were not significantly affected by the treatment.

Following the treatment with „Divaccon", the SCC increased more than twofold after the initial injection as compared to the values before the treatment. Only after the next two injections did the SCC decrease. After each application, levels of lactose (tab. 2) increased. Milk total proteins and milk freezing point were not significantly affected by the treatment.

A distinct SCC decrease was observed in treating udder quarters with „Leocillin" (tab. 1) intramuscularly. However only 40% of cases were cured. In cured

Tab. 1. Changes of somatic cells count (SCC) during the process of treatment of subclinical mastitis in cows

Preparation	Somatic cells count ($10^3/cm^3$)			
	Before treatment	After the first inject.	After the second inject.	After treatment*
Leo Yellow	2046.30 ± 228.31	1266.50 ± 89.35	831.43 ± 46.86	266.37 ± 25.00
Tetra Delta	1886.80 ± 223.55	1703.80 ± 274.72	1013.70 ± 140.14	277.00 ± 19.65
Mastijet Fort	2453.10 ± 245.54	2771.60 ± 377.65	3156.60 ± 324.98	375.94 ± 16.32
Divaccon	910.14 ± 81.94	2628.90 ± 564.43	1816.10 ± 570.38	457.86 ± 62.59
Mastisan A	1642.70 ± 285.37	3536.40 ± 379.94	3220.40 ± 422.95	471.21 ± 24.69
Leocillin	4166.00 ± 1177.2	1158.50 ± 304.56	993.67 ± 261.03	390.50 ± 44.16
Leo Yellow + Leocillin	3349.40 ± 954.52	1714.80 ± 413.01	1238.10 ± 322.32	258.71 ± 35.62

Explanation: * immediately after the end of witholding time

Tab. 2. Changes in the amount of lactose (%) during the process of treatment of subclinical mastitis in cows

Preparation	Lactose (%)			
	Before treatment	After the first inject.	After the second inject.	After treatment*
Leo Yellow	3.75 ± 0.084	4.08 ± 0.082	4.24 ± 0.096	4.58 ± 0.057
Tetra Delta	4.12 ± 0.074	4.27 ± 0.067	4.35 ± 0.061	4.51 ± 0.046
Mastijet Fort	3.93 ± 0.095	3.99 ± 0.107	4.14 ± 0.09	4.30 ± 0.065
Divaccon	3.75 ± 0.178	3.79 ± 0.126	3.96 ± 0.12	4.15 ± 0.102
Mastisan A	4.00 ± 0.111	3.68 ± 0.105	3.69 ± 0.11	3.86 ± 0.12
Leocillin	4.02 ± 0.109	4.15 ± 0.075	4.06 ± 0.066	4.49 ± 0.049
Leo Yellow + Leocillin	3.99 ± 0.142	4.19 ± 0.132	4.37 ± 0.104	4.41 ± 0.078

Explanation: * immediately after the end of witholding time

Tab. 3. Changes in the amount of total protein (%) during the process of treatment of subclinical mastitis in cows

Preparation	Total protein (%)			
	Before treatment	After the first inject.	After the second inject.	After treatment*
Leo Yellow	3.13 ± 0.058	3.15 ± 0.056	3.17 ± 0.047	3.16 ± 0.049
Tetra Delta	2.69 ± 0.045	2.84 ± 0.076	2.91 ± 0.07	2.86 ± 0.042
Mastijet Fort	2.86 ± 0.067	3.25 ± 0.096	3.33 ± 0.109	3.02 ± 0.076
Divaccon	2.88 ± 0.122	2.94 ± 0.159	2.96 ± 0.085	3.07 ± 0.139
Mastisan A	2.79 ± 0.063	2.75 ± 0.057	2.83 ± 0.057	2.81 ± 0.055
Leocillin	2.52 ± 0.049	2.55 ± 0.03	2.51 ± 0.039	2.48 ± 0.065
Leo Yellow + Leocillin	2.51 ± 0.074	2.67 ± 0.056	2.69 ± 0.037	2.65 ± 0.061

Explanation: * immediately after the end of witholding time

Tab. 4. Changes of the freezing point of milk (– °C) during the process of treatment of subclinical mastitis in cows

Preparation	Freezing point of milk (–°C)	
	Before treatment	After treatment*
Leo Yellow	0.534 ± 0.001	0.539 ± 0.001
Tetra Delta	0.526 ± 0.001	0.532 ± 0.001
Mastijet Fort	0.530 ± 0.001	0.530 ± 0.001
Divaccon	0.523 ± 0.003	0.529 ± 0.003
Mastisan A	0.529 ± 0.003	0.528 ± 0.001
Leocillin	0.526 ± 0.001	0.528 ± 0.001
Leo Yellow + Leocillin	0.527 ± 0.002	0.531 ± 0.002

Explanation: * immediately after the end of witholding time

quarters, lactose amounts (tab. 2) increased and the milk freezing point decreased. Milk total protein values were not significantly affected by the treatment.

Using a combined treatment of „Leo Yellow" in the affected quarter and „Leocillin" intramuscularly 93.33% of cases were cured. After three applications, SCC decreased (tab. 1), lactose amounts (tab. 2) and milk total proteins (tab. 3) increased, while the milk freezing point decreased (tab. 4).

Best effect for treatment had preparation Leo Yellow 88.71 ± 5.35% and Tetra - delta 79.49 ± 6.47%. Mastiject-Forte® 66 ± 6.6 % and Divaccon 28 ± 8.98%. By control preparation Mastisan A was treated 70 ± 10.25% quarters of udder. About influence of preparation on treatment we decide by changes in SCC, lactose, total proteins and milk freezing point. Some preparations (Mastiject-Forte®, Divaccon and Mastisan A) after injection into the udder irritate mammary gland and increasing SCC in it. Injection of Leocillini intramuscularly caused weaker antibacterial effect ($p < 0.001$). It demonstrates the studies carried out after the treatment. A direct effect of antibacterial preparations on the infected tissue is stronger. When combination of Leo Yellow and Leocillini was used for treatment of subclinical mastitis the effect has grown up to 93.33 ± 6.44%.

Our investigations show that subclinical mastitis cases are usually caused by mixed microflora – 52.85% and 47.15% by pure cultures. This data is in agreement with J. Siugzdaite (1997), S. Japertas (2000), M. Paape et all. (2002) and W. Wawron, M. Szczubial (2002).

Subclinical mastitis is caused by mixed pathogenic micro-flora and pure-cultured microbes. In treating subclinical mastitis it is important to select antibiotics that are effective against identified micro-flora. In using such preparations, the basal ingredient must not irritate udder tissue.

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SUTTORP M., HOFFMANN B., SIPPELL W. G.: Zapobieganie zatruciu spowodowanemu przez estradiol u dalmatyńczyka przez wczesne zastosowanie czynnika stymulującego kolonie granulocytów. (Prevention of oestradiol-associated toxicosis in a dalmatian by early intervention with granulocyte colony-stimulating factor). Vet. Rec. 151, 244-245, 2002 (8)

Egzogenne estrogeny w wysokich dawkach działają toksycznie na szpik kostny psów, powodując nawet zejście śmiertelne. To działanie myelotoksyczne jest następstwem uszkodzenia komórek pnia. W związku z dobrymi efektami korygowania granulocytopenii i stymulacji komórek pnia pod wpływem czynnika stymulującego kolonie granulocytów (G-CSF) zastosowano tę substancje u 14-miesięcznej suki dalmatyńskiej w fazie *anestrus*, która połknęła 30 tabletek zawierających 60 mg walerianu estradiolu i 21 mg norethisteronu. Po 29 godz. wystąpiła leukocytoza ($18{,}4\times10^9$ kom/l), wzrósł poziom estradiolu w plazmie krwi (254 pmol/l). W celu stymulacji granulocytozy zastosowano *per os* 2,3 mg prednizolonu/kg i 11,5 mg węglanu litu/kg w odstępach 12 godz. przez 3 doby, a następnie G-CSF w dawce 9,2 mg/kg w iniekcji podskórnej 2 razy dziennie. Następnie dawkę C-GSF obniżono do połowy 20. dnia i kontynuowano leczenie przez 16 dni. W 54. dniu leczenia parametry krwi osiągnęły wartości fizjologiczne. Pies w czasie leczenia był w bardzo dobrej kondycji. Nie stwierdzono żadnych niekorzystnych następstw zatrucia w czasie 20-miesięcznej obserwacji psa po leczeniu.

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