Electronic Journal of Polish Agricultural Universities is the very first Polish scientific journal published exclusively on the Internet, founded on January 1, 1998 by the following agricultural universities and higher schools of agriculture: University of Technology and Agriculture of Bydgoszcz, Agricultural University of Cracow, Agricultural University of Lublin, Agricultural University of Poznan, Higher School of Agriculture and Teacher Training Siedlce, Agricultural University of Szczecin, and Agricultural University of Wroclaw.

ELECTRONIC JOURNAL OF POLISH AGRICULTURAL UNIVERSITIES

2003
Volume 6
Issue 2
Series VETERINARY MEDICINE

MOLENDA J., TWARDOŃ J., BŁASZKOWSKA M., CZERW M. 2003. THE MICROBIOLOGICAL MONITORING OF PRODUCTION, GAIN, TRANSPORT AND PRESERVATION OF MILK **Electronic Journal of Polish Agricultural Universities**, Veterinary Medicine, Volume 6, Issue 2.
Available Online http://www.ejpau.media.pl

THE MICROBIOLOGICAL MONITORING OF PRODUCTION, GAIN, TRANSPORT AND PRESERVATION OF MILK

Jerzy Molenda[1], Jan Twardoń[2], Małgorzata Błaszkowska[2], Małgorzata Czerw[1]
[1]*Department of Animal Product Hygiene, Agricultural University in Wrocław, Poland*
[2]*Deparment of Animal Reproduction and Obstetric Clinic, Agricultural University in Wrocław, Poland*

ABSTRACT
INTRODUCTION
MATERIAL AND METHODS
RESULTS
DISCUSSION
CONCLUSIONS
REFERENCES

ABSTRACT

The purpose of the study was determination of microbiological contamination of dairy farms with a special regard of pathogenic bacteria, the causal agent of udder infection. Studies were carried out in two farms with various systems of maintenance and milking. Moreover, occurrence of different clinical form of mastitis in dairy cows and their causative agents were analysed. In the air of cow shed a lot of microorganisms were found, but without main udder pathogens, responsible for mammary gland in those farms. However, these pathogenic bacteria were many times isolated from dairy equipment /milking machines and containers/. The equipment then can be source of the pathogen and mediatory factor of its dissimination.

Among mammary gland infections subclinical mastitis, provoked with S.aureus, was the most often recognized. About half of total number of the strains produced enterotoxins. The coagulase-positive staphylococci were present too in collected milk, in both farms and for this reason the milk may be patential cause of food poisoning in humans.

Key words: microbiological monitoring of dairy farm environment, mastitis examination, udder pathogens, collected milk quality

INTRODUCTION

The requirements concerning the production and the bringing into UE market row milk are very high. They are included in a directive CD92/42, 1992 [13]. That directive determines conditions for bringing into UE market food products from countries that are not in the EU. Generally those products must ensure the same safety standard for consumers as products made in EU. So, imported row milk, exposed to thermal process and its products must fulfill the same conditions as UE products.

The directive determines requirements and conditions that should be fulfilled by milk producers on cow barn level. Milk must come from cow barns, which are veterinary tested at regular intervals, from cows in herds officially recognized as free from brucellosis and tuberculosis. Moreover the animals should not suffer from diseases for which milk could be a vector transmitting them to people (listeriosis, pyogenic bacteria, E. coli 0157 and others). Milk cannot also come from cows with alimentary canal disorders or mastitis. The same rules apply to administration of drugs and other chemicals that could be dangerous for human health.

According to that directive milk must be chilled to 8°C directly after milking (if the milk is taken away every day), or at least to 6°C (if it is taken away on the next day). The temperature of transporting milk can not be higher than 10°C. Storage tanks and milk containers must be washed and disinfected at least once a day and milking machines after each use. The stuff must comply with personal hygiene rules and have valid health control booklets. Milk, which is brought, or processing cannot have more than 100 000 of microorganisms and not more than 400 000 of somatic cells in 1 ml. It means that the directive gives definite requirements not only for milk companies but also for the whole system; that includes cows, cows' farms, milk's transport, milk processing and milk storage.

In Poland the requirements for purchasing row milk are defined in a standard: PN-A-86002 [14] legislated in November 1985 and amended in February 1999. That rule partly fulfils parameters of the number of microorganisms and somatic cells in 1ml of milk, that issue from the directive. The norm commands the investigations of milk producing herds with regard to general number of bacteria (2 times a month) and count of somatic cells (once a month). But, it has no full program for a supervision of milk production on cow barn level, that would include also the udder health control, registry of etiological factors of mastitis and the environmental influence on them.

The aim of our investigations was to obtain information of the cows' health and udder's condition that influenced milk quality, cow barn level, estimated in microbiological examination. We considered environmental conditions in milk gain, it means: type and sanitary state of a cow barn, way and hygienic conditions of milking and milk preservation and transport.

MATERIAL AND METHODS

The investigations were made between August 1999 and December 2000 in two cow barns with various systems of living and milking. Cow barn B has 210 HF cows in age 3 to 7 years. Cows were held in leashes in traditional, long bays. The animals were milked in bays by De-Laval canal system. During milking the milk is sent to milk chillers in separate room and chilled to 4°C. Cows with high milk efficiency (about 8100 liters milk per year) are milked two times a day. Milking machines are automatically washed with changeable pH hygienic medium. Moreover in few days milking machines are taken away and washed by hand. Liners are changed after 6 months. Each teat is washed after milking. Milk is taken away to a creamery every day by a milk truck. Generally it can be said that in this cow barn basic hygienic rules of milking and milking machines are kept.

The second cow barn S has 70 cows, held without bays. Cows are milked twice a day in a milking parlour (parallel 2x4 type) made by De Laval. Milk is chilled to 4°C in a 4000 liters milk container. Milking machines are automatically washed with a proper hygienic medium. Living and feeding status of cows was good. Average efficiency is 5300 liters of milk per year.

Health state of udder was estimated by clinical investigation of mammary gland, the counting of somatic cells (TOK method) and bacteriological investigations on milk samples. These investigations were made for every cow beginning from dry period (8-6 weeks before delivery) and 10-14 days after delivery. On the ground of those examinations we selected two groups of cows. The first one – healthy cows (mammary gland and milk without changes) and the second group – infected cows (various changes in udder tissue and milk).

Material for bacterial examinations:

1. Swabs fro surfaces of:milking machines and storage tanks (cans, tanks in milking parlor, transported tanks).
2. Milk samples.
3. Examinations of air in cow barns, milking-machines parlor and milk collectors.

The samples were collected from two milk farms with different technological systems, in summer and winter season.

Bacteriological examination was qualitative and quantitative. Blood medium supplemented 5% sheep blood and Mc Conkey Agar were used.

Identification of bacteria was made by using standard analysis tests with biochemical, physiological and colony morphological features. API 20E, API STAPH, API 20STREP, API 20C, API-LAB PLUS (bioMerieux) were used. The CF factor in cell wall was detected by Cornay St-80 reagent and free coagulase by lyophilized rabbit serum (Biomed). Group antigens in Streptococcus were detected by Slidex Streptp Kit (bioMerieux).

The number of living microorganisms in milk samples was detected by examination of 1 ml of milk and its next dilution. It was made with using:

- Petrifilm AC for aerobic bacteria;
- Petrifilm CC for coli-forms

The number of bacteria was estimated approximately by counting colonies, which grew up on Petri dish with blood medium supplemented 5% sheep blood.

Species of bacterium was recognized by colony morphological features and normal diagnostics proceedings.

The number of microorganisms in the air were examined by 15 min exposition of opened dishes with blood medium supplemented 5% sheep blood. The plates were placed on the floor, and on the 1m height.

Enterotoxin production by Staphylococcus aureus strains we had found, were examined with Tetra Staphylococcal Enterotoxins.

RESULTS

We made: clinical investigations of cows and bacteriological investigations of:

- the air in cow barns and storage tank rooms (29 samples);
- the swabs from milking-machines and milk containers surfaces (69 samples);
- milk samples from cows with TOK positive test (185 samples);
- milk samples from milk collectors (7samples).

In the air a lot of microorganisms were found, but their number didn't change in winter and summer significantly. There were different species of bacteria and fungi, but without main udder pathogens: Streptococcus. agalactiae and Staphylococcus sp (Table 8). The air in milking parlor and in storage tank rooms was much more better than in cow barns (the number of microorganisms were 10-20 times lower). But in one of milking parlor pathogenic staphylococci. were found (Table 1).

On both farms typical udder pathogens were found on teat cups, storage tanks and transport tanks. The high frequency of finding these typical udder pathogens showed insufficient quality of washing.

In 69 samples from milking machines and milk containers surfaces 6 times Str. agalactiae and 12 times Staph. aureus were found. On both farms the number of pathogen free samples is similar suggested insufficient quality of automatic wash system (Table 2).

Table 1. Cow barn environment contamination by pathogenic bacteria

The place of Investigation	S. aureus	Farm S S. agalactiae	E. coli	S. aureus	Farm B S. agalactiae	E. coli
ANIMALS udder skin	–	–	–	+	–	–
STUFF hands skin	–	–	–	–	–	–
AIR	+	–	+	–	–	+

Table 2. Contamination of milking machines and milk containers with pathogenic bacteria

Machines	Farm S.			Farm B.		
	S. agalactiae	S. aureus	Number of samples	S. agalactiae	S. aureus	Number of samples
Teat cups	2	1	14	2	5	17
Storage tanks	1	2	6	1	1	11
Milk transporter	0	2	4	0	0	8
its pipe	0	1	4	0	0	5
ALL	3	6	28	3	6	41

Milk samples from cows with TOK positive test were examined in order to find etiological mastitis factors. The results are in tab.6. These results show that the greater number of mastitis was caused by Staphylococci pyogenic. It was showed that the most of infectious Streptococci and Staphylococci were isolated from subclinical infections, which were not recognized by stuff and milk from those cows was very often collected together in storage tanks with milk from healthy cows.

On the ground of udders investigations in cow barn B we noticed 101 disorders with different intensity. In 101 cows 81 had subclinical and the rest clinical mastitis. In clinical mastitis 4 were acute and 16 chronic mastitis with various intensity of pathomorphological changes (tab. 5).

In clinical examination 64 cows had disorders in teat canal and its spot. Those were: the issue fractures or loses of mucosal membrane. They could cause inflammatory disorders of udder.

On farm S 30 cows had inflammatory disorders (infectious or noninfectious) of udder tissue, 24 cows had subclinical and 6 - clinical mastitis (Table 4). More than a half of pathological changes were caused by udder injures. Milking in a milking parlour significantly limited infectious udder disorders.

Are shown in tab. 4 the participation of pathogenic bacteria responsible for udder diseases.

In cow barn B in milk samples from 58 cows Staphylococcus aureus was found, Streptococcus agalactiae in 20 milk samples, in 4 – Arcanobacterium pyogenes and E. coli in 9 milk samples (Table 5).

In cow barn S in 24 milk samples Streptococcus aureus and in 2 E. coli were found. In next 4 milk samples from cows with mastitis no bacteria were found, it shows the aseptic mastitis.

On both farms the main etiological factor of mastitis was Streptococcus aureus (more than a half of the cases). On farm S, in the opposition to farm B, no other etiological factors of mastitis were found. Lower number of mastitis in cow barn S could be caused by different living, milking and management systems (Table 3).

Table 3. The results of bacteriological examination samples of milk in farm B

Season	Number of samples	Samples with			Pathogenic bacteria	%*
		no growth bacteria	growth	pathogenic		
Autumn	77	29	48 62.3%	46 59.7%	S. aureus S. agalactiae A. pyogenes	95.6%
Winter	61	41	30 49.2%	19 31.1%	S. aureus E. coli	63.3%
Spring	15	3	12 81.3%	6 40.0%	S. aureus S. agalactiae	50.0%
Summer	42	16	26 61.9%	21 50.0%	S. aureus S. agalactiae A. pyogenes	80.8%
ALL	**195**	**89**	**116**	**92**		

* - percent of samples with growth of pathogenic bacteria in all samples contaminated with microorganisms

Table 4. The results of bacteriological examination samples of milk in farm S

Season	Number of samples	Samples with			Pathogenic bacteria	%*
		no growth bacteria	growth	pathogenic		
Winter	60	43	17 28.3%	15 25.0%	S. aureus	88.0%
Autumn	30	8	22 73.3%	11 36.7%	S. aureus E. coli	50.0%
ALL	**90**	**51**	**39**	**26**		

* - percent of samples with growth of pathogenic bacteria in all samples contaminated with microorganisms

Table 5. The number of cows with different forms of mastitis caused by pathogenic microorganisms

Clinical type of infection	FARM B					FARM S		
	Number of Investigated cows	Cows with				Number of investigated cows	Cows with	
		S. aureus	S. agalactiae	E. coli	A. pyogenes		S. aureus	E. coli
Subclinical mastitis	81	48	15	6	1	24	20	0
Acute mastitis	4	2	0	0	2	1	1	0
Chronic mastitis	16	8	5	3	1	5	3	2
ALL	**101**	**58**	**20**	**9**	**4**	**30**	**24**	**2**

Table 6. Number and species of bacteria in milk samples from cows with very high number of somatic cells

Number of sample	Number of somatic cells	Number of bacteria	Kind of microorganisms
1	3460 000	$2.9x10^5$	S.aureus (67cfu)*, E.coli (42cfu), Saprophitic organisms
2	5820 000	$1.7x10^5$	S.aureus (pure culture)
3	9900 000	$4.7x10^4$	S.aureus (12cfu), Gramm-negativ rods (18cfu)

The results in milk samples from milk collectors (an average number of 3 determinations of each sample) showed that the number of bacteria was, in about half of the samples, higher than 100 000 cfu/1ml. Two samples (one from each farm) contained pathogenic staphylococci, wich however were unable to produce enterotoxins. On one farm (S) this sample was taken in summer and its micobiological contamination raised to $>10^6$ bacterial cells in 1 ml. On farm B pathogenic staphylococci were found in autumn season and the sample contained a little bit more microorganisms than 60 000 cfu/ml. In these samples: of collected milk Micrococcus sp, Streptococcus bovis, S. lactis, Aerococcus, Coliforms, Enterobacter sp, Aeromonas sp. Actinobacter were found. Those are microorganisms, which lead to spoilage of food (Table 7).

Table 7. Bacteriological investigations of milk in milk container

FARM	Season	Number of sample	Number of somatic cells	Number of bacteria	Kind of microorganisms
S	Winter	1	Notinvestigated	$4.9x10^3$	Micrococcus Aerococcus S. bovines
	Summer	1	Not investigated	$>10^6$	S.aureus Gramm-negativ rods Saprophitic Gramm-positive
B	Summer	1	Not investigated	$4.8x10^4$	Saprophitic Gramm-positive
	Autumn	1	Not investigated	$6.3x10^4$	S.aureus Gramm-positive rods Gramm-positive cocci
		1	173 000	$>10^6$	Gramm-negativ rods Saprophitic Gramm-positive
	Winter	2	381 000	$4.3x10^5$	E.coli Gramm-positive rods Enterococci
		3	498 000	$2.7x10^5$	E.coli Gramm-negativ rods Saprophitic Gramm-positive

Table 8. List of microorganisms isolated in the examination

MORPHOLOGY	SPECIES/GENUS
Cocci Gramm-positiye	Micrococcus lysodeiticus Micrococcus luteus Micrococcus varians Staphylococcus aureus Staphylococcus epidermidis Staphylococcus haemolyticus Streptococcus agalactiae Streptococcus bovis Streptococcus lactis Streptococcus faecalis Streptococcus durans Streptococcus mitis Aerococcus viridans
Gramm-positive rods	Microbacterium lacticum Microbacterium thermosphactum Propionibacterium
Spore-forming bacilli	Bacillus subtilis Bacillus licheniformis Bacillus mesentericus Bacillusmegaterium
Moulds	Aspergillus sp Penicilium sp. Fusarium sp.
Yeasts	Candida sp. Torulopsis sp. Cryptococcus sp.
Gram-negative rods	Escherichia coli Enterobacter cloaca Enterobacter agglomerans Citrobacter freundi Aeromonas caviae Aeromonas sobria Pseudomonas sp. Acinetobacter sp.

Enterotoxin production by. coagulase-positive staphylococci is the main threat for human health. On these farms we found in many cows subclinical udder infections, caused just by pyogenic staphylococci. By that reason and because of their presence in milk collectors, we decided that it is necessary to determine their enterotoxic features in our isolated staphylococci strains. So, there were 41 strains (27 from udder infections, 14 from swabs of milk equipment). About a half (20 with udder, 6 from swabs) were able to produce enterotoxins. It shows that milk and its products could be potentially dangerous for human health.

DISCUSSION

Microbiological investigations of cow barn's environment, milk containers, milking machines, storage tanks and milk transporters showed bacteria, which were recognized as mastitis' etiological factors. Those bacteria were more often found on milking machines and storage tanks. They were more often found in cow barn B. It shows that milk could be contaminated, or the disinfections and wash efficacy were not enough good. It is obvious that the presence of microorganisms in environment or on machines caused the spread of cows diseases. It was proved that size of liners could cause udder disorders, because during the washing bacteria could resist in micro rifts of a liner [11,12]. Improper technical quality of milking machines caused disorders in udder balance [12].

Animals are the reservoir of microorganisms. The clinical investigations of udder, and bacteriological examinations of milk samples showed that most of diseases were subclinical. That form of infection dominates in milking cows, sometimes affects about 60% of animals in one herd [1,2,6,9]. The losses caused by that are significant, because their expenses amount about 70% of all losses in milk production on cow barn level.

In about half of the cows with subclinical mastitis Staphylococcus aureus was found. That microorganism is very often a cause of subclinical udder disorders [2,4,5,6,7,8,9,10]. Subclinical mastitis has no clinical signs, and there is nothing to show that the cow should be removed from production. In the result, microorganisms responsible for subclinical disorders penetrate to the collected milk. According to concentration and virulence of those bacteria and way of milk processing, milk and milk products can become infective for people. In this case it is very important to chilly the milk as quickly as possible and to keep proper temperature during transport. E. coli strains those were isolated in our investigations, did not belong to verotoxic serotype 0157; H7 that is very dangerous for human health.

In quantitative examinations, pathogenic staphylococci in milk samples from cows with subclinical mastitis were calculated between $2.3x10^2 – 1.6x10^4$ cfu/1ml. In spite of the fact, that the bacteria were quite often found in single milk samples, we found them only once in the collected milk. But the collected milk was investigated only a few times, so it is hard to estimate the state of emergency. However, we decided that it was necessary to define enterotoxic features of isolated strains. Among 41 of the strains, 20 revealed the ability to produce enterotoxins. Then milk and its products can contain pathogenic staphylocicci and for this reason they can be able to cause food poisoning in humans.

As for as the hygienic status during the milk gain is concerned, general number of microorganisms in milk was testified. Lower number of microorganisms was found in winter.

We would like to show that general number of microorganisms in milk samples transported directly after collection to laboratory was lower than it should be according to the law PN-A-86002 and the directive 92/46 EEC for "extra" class (100 000 = 10^5 microorganisms in 1ml of milk). But that criterion was not always fulfilled and caused the exception of milk from those farms from "extra" to lower classes. The explanation of this problem needs further investigations.

CONCLUSIONS

1. In environmental examinations pathogenic microorganisms were found on milking-machines cans and in milk collectors. Sometimes on udder skin and in streak canal, too. Typical udder pathogens were never found on the stuff hands. They were found only ones in the air of milking parlor.
2. Most of udder infections occurred in subclinical form.
3. Pyogenic staphylococci (S. aureus strains) were the most responsible for disease, more often they are the only cause on farm S and dominant cause on farm B.
4. In both farms we found insufficient quality of washing and disinfection. It could cause the high number of microorganisms in some milk samples from milk collectors.

5. About a half of tStaphylococcus aureus strains were able. to produce enterotoxins. It could be reason of food poisoning in humans.
6. On the basis of our results it looks indispensable to include milk production supervision in cow barns especially with monitoring udder health and hygiene of the milk gain and include accustoming of GMP rules in the milk production process.

REFERENCES

1. Bosteadt H., Boryczko Z., Sheid T., 2001. Diagnostyka i terapia ostrych postaci zapalenia gruczołu mlekowego., [Diagnosis and therapy of acute mastitis] Życie Wet., 76, 477-479 [in Polish].
2. Busato A., Trachsel P., Schallbaum M., 2000. Udder health and risk factor for subclinical mastitis in organic dairy farms in Switzerland., Prev. Vet. Med., 44, 205-220.
3. Bystroń J., Nosek-Paszkowska K., Molenda J., 2001. Występowanie gronkowców enterotoksycznych w mleku surowym., [Occurrence of enterotoxigenic staphylococci in raw milk]. Medycyna Wet. 57, 645-648 [in Polish].
4. Chaffer M., Leitner G., Winkler M., 1999. Coagulase-negsative Staphylococci and mammary Gland infections in cows., J. Vet. Med. B., 46, 707-712.
5. Czupa S., Czupa M., 2001. Laboratory diagnostic of mastitis in the cows., Życie Wet., 76, 471-473.
6. Hillerton J.E., Walton A.W., 1991. Identification of subclinical mastitis with a handheld electrical conductivity meter., Vet.Rec., 128, 513-515.
7. Malinowski E., Kłossowska A., 1999. Mastitis in pregnant heifers., Medycyna Wet., 55, 651-654.
8. Nickerson S.C., 1993. Eliminating chronic Staphylococcus aureus mastitis., Vet. Med., 88, 375-381.
9. Reinard P., Poutrel B., 1982. Dynamics of nonclinical bovine intramammary infections with major pathogens., Am.J.Vet.Res., 43, 2143-2146.
10. Thompson P.D., 1977. Effects of physical characteristics of milking machines on teats and udder., J.Am.Vet.Med.Assoc., 170, 1150-1154.
11. Twardoń J., Dejneka G.J., Dzięcioł M., 2001. Milking mashines as the potential source of udder infections., Życie Wet., 76, 651-654.
12. Wawron W., 2001. Occurrence, diagnosis and treatment of subclinical mastitis in cows., Życie Wet., 76, 473-476.
13. Council Directive 92/46/EEC if 16 June 1992 laying down the health rules from the production and placing on the market of the raw milk, heat-treated milk and milk based products.
14. PN – A – 8602 (luty 1999). Mleko surowe do skupu. Wymagania i badania. [Purchase of raw milk Requirements and examinations. February 1999] [in Polish].

Jerzy Molenda, Małgorzata Czerw
Department of Animal Product Hygiene
Agricultural University in Wrocław
C.K. Norwida 31, 50-375 Wrocław, Poland
Ph (71) 32-05-265
Jan Twardoń, Małgorzata Błaszkowska
Deparment of Animal Reproduction and Obstetric Clinic
Agricultural University in Wrocław
Pl. Grunwaldzki 49, 50-366 Wrocław, Poland
Ph (71) 32-05-306
