Technologically difficult, pathogenic and food risky bacterial contamination of raw milk and other materials from dairy cow herds

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ABSTRACT: Processing of milk for higher value-added products and milk food chain safety require prevention of higher occurrence of microorganisms that can produce spores. It is important to carry out prevention already during raw milk production by farm hygienic measures. The above-mentioned microorganisms can jeopardize the quality and safety of milk foods. This is the reason why the knowledge of factors, interrelationships and possibilities of milk contamination is assumed. The occurrence of hygienically important groups of microorganisms in mixed rough fodder (F) in feeding trough, in excrements (E) and in bulk milk (M) was investigated on seven farms with a good level of milk yield, management and hygienic conditions for three years in summer (S) and winter (W) feeding seasons. The data set was well balanced in terms of other possible farm factors (such as height above sea level, breed of dairy cows and so on). In total 70 samples of each mentioned material were collected. The investigations were focused on counts of thermoresistant bacteria (TRB), total counts of bacilli (TBA), frequency of occurrence of *Bacillus cereus* (BCE) and *Bacillus licheniformis* (BLI), and counts of other bacilli (OBA) in all mentioned materials and on the frequency of occurrence of main mastitis pathogens *Streptococcus agalactiae* (SAG) and *Staphylococcus aureus* (SAU) in milk. The means of parameters did not exceed the defined standard limits, in particular in raw milk that was suitable for direct human consumption in raw state or for processing for milk products without heat treatment according to relevant EU food legislation. The variabilities of hygienic parameters were relatively high: from 179% (TBA) to 315% (TRB) in F; from 178% (BLI) to 350% (TRB) in E; from 117% (TBA) to 459% (SAG) in M. S increased TRB in F ($P > 0.05$). Counts of bacilli were well balanced between S and W. S increased BLI in E ($P \leq 0.05$), an opposite trend was observed in BCE. The season did not influence SAG and SAU in M. Higher ($P \leq 0.01$) counts of BCE were in S, the trend was opposite for BLI ($P \leq 0.05$). The season effect on the occurrence of technologically and hygienically difficult microorganisms was not confirmed in general for M, F and E of dairy cows. Regression analysis of the investigated parameters was done. There were significant positive correlations between bacilli and TRB in F as well as in E (correlation coefficients from 0.32 to 0.65; $P \leq 0.01$). The relationships between TRB and occurrence of bacilli in M were less close (0.30; $P \leq 0.05$) than in F and E. No significant relationships were found between TRB and occurrence of main mastitis pathogens in milk (SAG $r = -0.14$ and SAU $r = 0.11$; both $P > 0.05$). It confirmed the high pasteurization efficiency at liquidation of mentioned pathogens. The relationship SAU × SAG in M was 0.23 ($P < 0.05$). It suggests a simultaneous effect of dairy cow health state on the occurrence of both pathogens. The majority of significant relationships (from 0.26 to 0.76; from $P \leq 0.05$ to $P \leq 0.001$) was in F × E within TRB, BLI, OBA and TBA. 7% to 58% of the changes in E were dependent on changes in F. The occurrences of TRB and BLI in M were significantly influenced (0.24 and 0.32; $P \leq 0.05$ and $P \leq 0.01$) by their occurrences in E and F. The observed dependences confirm the relationships between materials (F, E and M) and possibilities of penetration of TRB and bacilli through the technological chain along the axis F – E – M. The hygiene of rough fodder harvest and preservation is highly important for prevention of the above-mentioned risks.

Keywords: dairy cow; hygiene; rough fodder; excrements; milk; thermoresistant bacteria; total bacilli; *Bacillus cereus*; *Bacillus licheniformis*; other bacilli; food pathogens; mastitis pathogens; *Streptococcus agalactiae*; *Staphylococcus aureus*; food chain safety; prevention

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Hygienic risks of food technology. Most of milk is processed onto products after heat treatment, it means after pasteurization. Such a process involves many variants of performance in regard to efficiency in terms of temperature delicacy or height and time of treatment. A part of the original microbial contamination of milk can survive the pasteurization process as well, it is an important and well-known fact. The vegetative forms of microbes are destroyed in particular. Bacteria that form the spores are able to survive very often in their generative form. It is a very well-known fact and brings incidental risks into the milk food chain and from this aspect the original hygienic quality of raw material is quite a basic factor (Hanuš *et al*., 2001b). Vyletělová (2001) confirmed the penetration of thermoresistant sporulated microorganisms (bacteria, *Bacillus cereus* and *Bacillus licheniformis*) even through the UHT method of milk processing (138°C for 4 seconds).

The sporulated bacteria or their technological groups can incidentally cause the food destruction and defectiveness by their protease and lipase production and degradation procedures in milk products and can also reduce milk technological recovery, for example in cheese-making (Rowe and Gilmour, 1985; Shelley *et al*., 1986; Griffiths and Phillips, 1990; Ternström *et al*., 1993; Larsen and Jörgensen, 1997; Salkinoja-Salonen *et al*., 1999; Christianson *et al*., 1999; Vyletělová *et al*., 1999a,b, 2000a,b). Furthermore, some microorganisms (*Staphylococcus aureus, Bacillus cereus*) can cause alimentary health disorders in human consumers by their production of metabolic toxins.

Definition of maximal admissible hygienic limits. The above-mentioned facts are reasons why the microorganisms are limited by relevant food legislation which defines the legal basis for hygienic checks of food and food raw material quality. The observation of hygienic limits is continually checked because the opposite state could jeopardize the safety of milk food chain.

Basic European directive (EEC 92/46; EC 853, 2004) lays down the SAU (*Staphylococcus aureus*) ≤ 500 CFU/ml. The Czech Standard ČSN 57 0529 lays down the TRB (thermoresistant bacteria) ≤ 2000 CFU/ml as an additional parameter for extra raw milk quality. Ticháček and Benda (1991) and Ticháček *et al*. (1996) defined SAU ≤ 200 and SAG (*Streptococcus agalactiae*) ≤ 100 CFU/ml as parameters for a consolidated herd of dairy cows in terms of mastitis health state. According to their further results it meant the prevalence estimation of infectious mastitis ratio of relevant aetiology equal to 20.5% and 7.9% in the herd (Benda *et al*., 1997).

In the case of risky TRB it usually means sporulated facultative anaerobic and aerobic bacilli and anaerobic clostridia that very often come from fields of fodder crops and surfaces of rough fodder. The counts of so called mesophilic psychrotrophic aerobic sporulates (MPAS, mostly bacilli) ≤ 200 CFU/ml are limited for specific dairy technology of production of drinking milk with prolonged shelf (storage) time in raw milk (Hanuš *et al*., 2001b; Standards TEI 118 and EOL 103). This is a very exacting hygienic criterion. It was met in 53.8% of cases of raw milk deliveries for processing in the investigated consolidated area in terms of quality.

General conditions of important hygienic contamination occurrence in raw cow milk. The knowledge of prevalence of the occurrence of mentioned microorganisms in farm practice conditions is very important for special processing technologies. While the total counts of mesophilic microorganisms in milk are linked first of all with the hygiene of the milking process, the TRB occurrence would usually be connected with the spectrum of breeder technologies, for instance with the hygiene at harvest and preservation of rough fodder. Petersen (1985) and Andersen and Jensen (1987 – cit. Kratochvíl, 1991) pointed to the fact that microbial spores could penetrate into milk from excrements in the proximity of mammary gland. They come to this space by the digestive tract of dairy cows from contaminated preserved rough fodders of low quality, especially from grass silages that were harvested and preserved in a wrong way. The correlations of sporulate occurrence between materials such as feed × excrements, excrements × milk and feed × milk were unusually close and or else 0.80, 0.73 and 0.69. In the similar continuity Vyletělová (2001) reported the values of less close correlations (0.35 – $P < 0.01$, 0.28 – $P < 0.05$ and 0.23 – $P > 0.05$) for the relationships feed × excrements, excrements × milk and feed × milk. Nevertheless, the feed impact was confirmed as well.

The aim of investigation into the occurrence of sporulated microorganisms in raw milk and other related materials from dairy herds. The above-mentioned facts about technological importance and safety hygienic risks of sporulated bacteria occurrence were reasons for performance of selection of investigated microorganisms or microorganism groups in raw bulk milk, rough fodders (TMR = total mixed ration) and dairy cow excrements. The aim of the study was to evaluate the technological significance and to search for links between sporulated microorganism groups in different materials in order to explain and improve the prevention of occurrence of chosen microbial contaminants of raw milk.

MATERIAL AND METHODS

Structure of investigation. During three years of investigation the bulk milk samples were obtained in seven dairy cow herds. The samples were taken regularly in summer (August, September) and winter (February, March) seasons. The feeding types characterized the nourishment profiles in main agricultural production areas in the Czech Republic. Rough fodders were included in dairy cow rations for individual herds as follows: lucerne silage + maize silage; clover (clover and grass) silage + maize silage; grass silage + maize silage; pasture (summer), grass silage (winter) + GPS from cereals (or maize silage or sugar beet chips).

Three herds were Holstein breed (H) and three herds were Czech Pied cattle (C). The seventh herd comprised animals of both breeds C and H. The milk yields of investigated herds were from 5 500 to 10 000 kg of milk per cow and standard lactation. The milk yields of these herds were at average and above-average level of milk yield under the Czech Republic conditions.

The dairy cow herds were kept in lowland (L = 215 to 350 m height above sea level, three herds) and sub-mountain or mountain (M = > 350 to 605 height above sea level, four herds) areas. The proportions of animals of both dairy breeds (H and C) were well-balanced in the data set and in terms of the height above sea level as well.

Investigated hygienic and microbiological parameters of bulk milk, feeds and dairy cow excrements. The microbiological investigations were performed in an accredited testing laboratory of RICB Rapotin. The bulk milk samples were taken into sterile bottles. The corresponding samples of applied dairy cow ration (TMR) were taken from feeding troughs and samples of animal excrements from stable environment. All samples were frozen (–18°C) after cold transport (< 10°C). Analyses were performed after dissolving in unpreserved bulk milk samples and in mean feed and excrement samples for the following parameters:

– milk: all parameters are expressed in CFU/ml; TRB = thermoresistant bacteria; SAG = *Streptococcus agalactiae*; SAU = *Staphylococcus aureus*; BCE = *Bacillus cereus*; BLI = *Bacillus licheniformis*; OBA = other bacilli; TBA = total bacilli (mostly aerobic thermoresistant sporulated microorganisms)
– feed: similarly like in milk TRB, BCE, BLI, OBA and TBA, the results are expressed in CFU/ml of the solution that was prepared by dilution of 1g of material in 100 ml of distilled water
– excrements: the same way of analyses as in feed

Used microbiological cultivation methods:

1. The counts of thermoresistant bacteria (TRB) were determined according to Standard ČSN 57 0101 (the chapter Determination of Spore-forming Aerobic Bacteria Counts). The colonies of mesophilic bacteria species were counted after their growth for 72 hours of incubation at 30°C in milk samples that were previously inactivated by heating at 85°C for 10 minutes.

2. The *Staphylococcus aureus* (SAU) counts were determined according to Standard ČSN EN ISO 6888–1 (Horizontal Method for the Enumeration of Coagulase-positive Staphylococci – *Staphylococcus aureus* and Other Species – Part 1: Technique using Baird-Parker agar medium). It was performed by aerobic cultivation at 36°C for 48 hours. The confirmation was based mainly on a positive coagulase reaction.

3. The determination of *Streptococcus agalactiae* (SAG) counts was done according to Standard ČSN 57 0101 (the chapter Evidence of the Presence of Pathogenic and Opportunistic Pathogenic Microbes and Microbial Toxins).

4. The determination of *Bacillus cereus* (BCE) counts was done according to Standard ČSN ISO 7932 (General Guidance for the Enumeration of *Bacillus cereus* – colony count technique at 30°C).

Statistical evaluation. The basic statistical characteristics were calculated (using the programme Microsoft Office Excel 2003): arithmetical mean ($\bar{x}$); geometrical mean (x_g); standard deviation ($s_{\bar{x}}$); coefficient of variation (v_x in %); t values of t-test for evaluation of statistical significance of mean differences between the investigated effects.

The microbiological values were transformed logarithmically to obtain a possibility to work with geometrical means because arithmetical mean is not a suitable characteristic of frequency distribution of the values of microbiological parameters every time, because of usual deviation this distribution from normal (Ali and Shook, 1980; Shook, 1982; Hanuš *et al*., 1995, 2001a).

RESULTS AND DISCUSSION

Main characteristic of hygienic contamination results

The statistical evaluation of hygienic contamination of investigated materials from primary milk production is summarized in Table 1. High variability of the occurrence of all groups or species of investigated microorganisms is perceptible at first sight in all materials: feeds from 179% (total bacilli) to 315% (thermoresistant bacteria); excrements from 178% (*Bacillus licheniformis*) to 350% (thermoresistant bacteria); milk from 117% (total bacilli) to 459% (*Streptococcus agalactiae*). It is apparent that where variability (v_x) does not decrease below 100% everywhere, the less 35%, the geometrical means will be better statistical parameters or more reliable than arithmetical means because of no normal frequency distribution of original data sets. It confirms the competence of the used transformation of values for statistical testing.

As regards rough fodders (i.e. TMR = total mixed ration) and excrements, the obtained hygienic values (Table 1) are impossible to compare with any referential or discrimination legislative limits because no such limits exist. It is impossible to compare them with results in literature sources because of their sporadic occurrence in publications and because of the specific method of manipulation with the mentioned materials in our work. It means that these values can be compared and evaluated only in the framework of the results obtained in this work. On the contrary, the hygienic milk results are comparable with legislation limits or results of different papers.

Evaluating mutual relationships between the investigated microorganisms and groups of microorganisms (these are shown in scheme on Figure 1, just in the framework) which contaminate milk it is possible to introduce the following formulas: BCE + BLI + OBA = TBA; MPAS = ca. TBA ≤ TRB. The fact that physiological and technological definitions of the groups of microorganisms are partially overlap-

Table 1. Summary results of three-year investigations of selected microbial parameters that are risky for technology and consumer health in feed, excrements and milk samples in seven model dairy cow herds

Material	Statistical parameter	TRB	SAG	SAU	BCE	BLI	OBA	TBA
Feed	sample number (n)	70	69	70	70	70	70	70
	geometrical mean (x_g)	1 584.9			7.1	24.0	60.3	251.2
	arithmetical mean ($\bar{x}$)	8 726.4			51.6	405.7	603.7	1 061.0
	standard deviation ($s_{\bar{x}}$)	27 512.0			116.9	1 030.6	1 251.6	1 894.1
	coefficient of variation (v_x) %	315			226	254	207	179
	min.	23			0	0	0	0
	max.	208 000			600	5 300	8 500	8 500
Excrements	geometrical mean (x_g)	9 120.1			14.1	123.0	446.7	2 454.7
	arithmetical mean ($\bar{x}$)	35 166.4			125.6	1 457.9	5 415.7	6 999.2
	standard deviation ($s_{\bar{x}}$)	123 010.7			304.0	2 592.8	12 319.1	12 671.0
	coefficient of variation (v_x) %	350			242	178	227	181
	min.	90			0	0	0	0
	max.	1 000 000			2 000	15 000	83 000	86 100
Bulk milk	geometrical mean (x_g)	28.2	2.1	2.8	3.4	3.2	3.1	15.5
	arithmetical mean ($\bar{x}$)	75.6	33.5	17.8	12.9	9.2	11.9	34.0
	standard deviation ($s_{\bar{x}}$)	205.6	153.8	44.1	28.8	19.6	26.3	39.9
	coefficient of variation (v_x) %	272	459	248	224	212	221	117
	min.	0	0	0	0	0	0	0
	max.	1 600	1 050	220	170	140	150	170

Values in CFU/ml; CFU = colony forming unit; TRB = thermoresistant bacteria; SAG = *Streptococcus agalactiae*; SAU = *Staphylococcus aureus*; BCE = *Bacillus cereus*; BLI = *Bacillus licheniformis*; OBA = other bacilli; TBA = total bacilli

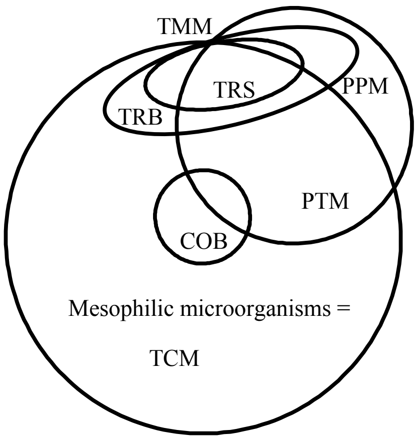

Figure 1. The pattern of possible schematic physiological and technological distribution of basic, standardized (Czech National Standard ČSN 57 0529) milk microbial contamination (modified according to Hanuš *et al.*, 2001)

TMM = total milk microflora
TCM = total counts of mesophilic microorganisms
PTM = total counts of psychrotrophic microorganisms
PPM = psychrophilic microorganisms
TRB = thermoresistant bacteria
TRS = thermoresistant sporulates
COB = coliform bacteria

ping is pointed by the mentioned schematic and facile relationships. *Bacillus cereus* can be considered as facultatively anaerobic and preferentially aerobic, sporulated, sometimes psychrotrophic, risky food pathogen (Hanuš *et al*., 2001b) at the same time.

The mean results did not exceed the differently defined discrimination limits (by legislation or by internal regulations on the basis of convention) for indicated microorganism groups, for instance for so called "extra quality milk" or milk from "healthy consolidated" herd (ČSN 57 0529, TRB ≤ 2000; EEC 92/46, EC 853, SAU ≤ 500; Ticháček and Benda (1991), SAG ≤ 100; standards EOL 103 and TEI 118, Hanuš *et al*. (2001b), MPAS ≤ 200, which is possible to compare to thermoresistant bacteria or in particular to total bacilli; all values are expressed in CFU/ml). It documents that milk from model herds corresponded to milk with the highest hygienic quality for a long time from the aspect of food safety checking.

Taking into account mean and maximal TRB values and especially the values of SAU and TBA (Table 1), all checked milk could be used for direct (raw milk) human consumption and for processing into milk products without heat treatment.

The impact of season on microbial contamination on investigated materials

Considering the presupposed main origin of sporulates in milk from preserved rough fodders (Petersen, 1985 and Andersen and Jensen (1987 – cit. Kratochvíl, 1991), Vyletělová (2001)) and considering possible practical effects of the phenomenon called secondary fermentation of these feeds, it is possible to formulate a hypothesis about the incidental effect of seasonal content of sporulated microorganisms in feeds, excrements and milk. The secondary fermentation is usually a consequence of no adequate picking of preserved rough fodders from their storages, in particular at higher environmental temperatures and at larger oxidation surfaces of feeds. It is often caused by shaking up of picked surfaces of preserved rough fodders or by leaving of rough fodders in interstorages, for example at direct sunshine. As it is also known, secondary fermentation is connected with an increase in contaminated microflora, with increased levels of undesirable microbial metabolites that can be toxic and with degradation of feeds and decrease in their nutrient contents. Therefore the secondary fermentation of feeds is dietetically and economically undesirable. The temperature increased by the season effect can support this phenomenon. As it is shown by results in Table 2:

– the summer season increased only slightly the counts of thermoresistant bacteria in feeds on feeding trough
– the counts of bacilli were essentially balanced between seasons
– the summer season increased substantially the *Bacillus licheniformis* counts in excrements, however an opposite trend was observed in *Bacillus cereus* counts, but it was less expressive. The other differences were not significant in excrements
– the season did not influence the main mastitis pathogen counts (*Streptococcus agalactiae* and *Staphylococcus aureus*) in milk. Only BCE counts were significantly higher during the summer season. The opposite trend was observed for BLI counts, however it was less expressive

In total, the results of the tests did not confirm the hypothesis about a possibility of season impact on the occurrence of microorganisms, which could be

Table 2. The results of testing the differences in the occurrence of selected microorganisms that are risky for technology and consumer health in different materials from dairy cow herds between summer and winter seasons during the period of three years in seven model herds

Material	Season	Statistical parameter	TRB	SAG	SAU	BCE	BLI	OBA	TBA
Feed	S	arithmetical mean ($\bar{x}$)	10 200.5			36.0	313.1	693.1	1 042.3
		geometrical mean (x_g)	1 621.8			6.6	19.5	67.6	263.0
	W	arithmetical mean ($\bar{x}$)	7 252.3			67.3	498.2	514.3	1 079.7
		geometrical mean (x_g)	1 584.9			7.6	28.8	53.7	239.9
	d	P	ns			ns	ns	ns	ns
Excrements	S	arithmetical mean ($\bar{x}$)	19 967.1			113.0	1 775.7	5 502.9	7 391.5
		geometrical mean (x_g)	8 511.4			28.2	281.8	371.5	2 511.9
	W	arithmetical mean ($\bar{x}$)	50 365.7			138.3	1 140.0	5 328.6	6 606.9
		geometrical mean (x_g)	10 000.0			6.9	53.7	537.0	2 454.7
	d	P	ns			*	*	ns	ns
Bulk milk	S	arithmetical mean ($\bar{x}$)	39.2	34.7	15.9	21.5	4.5	8.3	34.3
		geometrical mean (x_g)	26.3	2.0	2.4	6.2	2.2	2.3	17.4
	W	arithmetical mean ($\bar{x}$)	111.9	32.3	19.7	4.3	14.0	15.4	33.7
		geometrical mean (x_g)	30.2	2.2	3.2	1.9	4.5	4.2	13.8
	d	P	ns	ns	ns	**	*	ns	ns

Values in CFU/ml; S = summer; W = winter; d = difference; S = 35 samples, W = 35 samples; ns = not significant; *$P \leq 0.05$; **$P \leq 0.01$

difficult for technology and consumer health and pathogens in raw milk, feeds and excrements in dairy cow herds.

Relationships of microorganism occurrence within and between materials from dairy cow herds

The regression analysis of hygienic parameters was performed and correlation coefficients were calculated (Tables 3 and 4):

- significant positive relationships in feeds on feeding trough (Table 3) were observed between bacilli and thermoresistant bacteria, similarly in excrements, which has logical reasons. The mentioned fact shows striking occurrence of total bacilli (TBA) in the technological microbial group TRB. The changes in TRB counts were conditioned by changes in bacilli occurrence by 30% (Figure 2) to 42% (Figure 3)
- the relationships between TRB and bacilli occurrence were less narrow (Table 3) and less often demonstrable in raw milk than in feeds and excrements
- the absolutely no significant relationships between TRB and main mastitis pathogen occurrence in milk (SAG $r = -0.14$ and SAU $r = 0.11$, both $P > 0.05$) demonstrate a pasteurization efficiency (which was used in the method of TRB determination) at liquidation of these mastitis pathogens
- the positive relationship SAU × SAG in milk is remarkable (Table 3; $r = 0.23$; $P < 0.05$; Figure 4). It indicates simultaneous effect of herd health state on the occurrence of both pathogens. The increased mastitis pathogen occurrence was related to higher bacilli counts as well (Table 3)
- most of the significant relationships between microorganism occurrences were observed in the link feed × excrements (Table 4). The mentioned fact holds good for thermoresistant bacteria, *Bacillus licheniformis*, other bacilli, and total bacilli.

Table 3. Significant correlations between microbiological parameters in materials such as feed, excrements and milk

Material	Parameter	BCE	log BLI	OBA	log OBA	TBA	log TBA	
	TRB	0.54***						
	log TRB		0.45**		0.42**		0.65***	
Feed	BCE					0.28*		
	OBA					0.85***		
	BLI			0.33**		0.77***		
		BCE	BLI	log OBA	TBA	log TBA		
	TRB	0.33**	0.32**					
Excrements	log TRB			0.33**		0.55***		
	log BLI					0.30*		
	OBA				0.98***			
		SAU	BLI	log BLI	OBA	log OBA	TBA	log TBA
	SAG	0.23*			0.34**			
	log SAU			0.32**				0.28*
	TRB		0.28*					
Bulk milk	log TRB							0.30*
	BCE						0.58***	
	log BCE					–0.31**		
	BLI						0.45**	
	OBA						0.54***	

$^*P \le 0.05$; $^{**}P \le 0.01$; $^{***}P \le 0.001$

Their changes in excrements were dependent on changes of their occurrence in feeds by 7% to 58% (Figures 5 and 6)

– the occurrences of TRB and BLI were significantly influenced by their occurrences in excrements and feeds (Table 4; Figure 7)

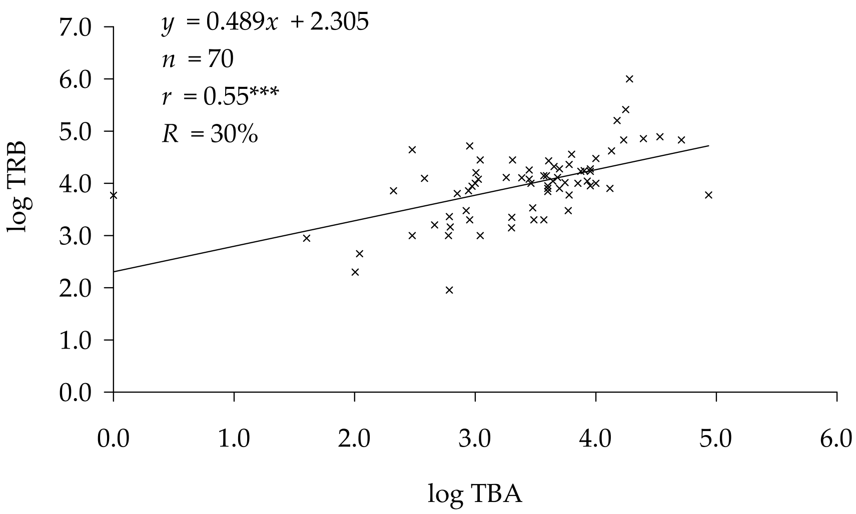

Figure 2. The relationship between the frequency of occurrence of bacilli (TBA) and thermoresistant bacteria (TRB) counts in excrements

r = coefficient of correlation
R = coefficient of determination

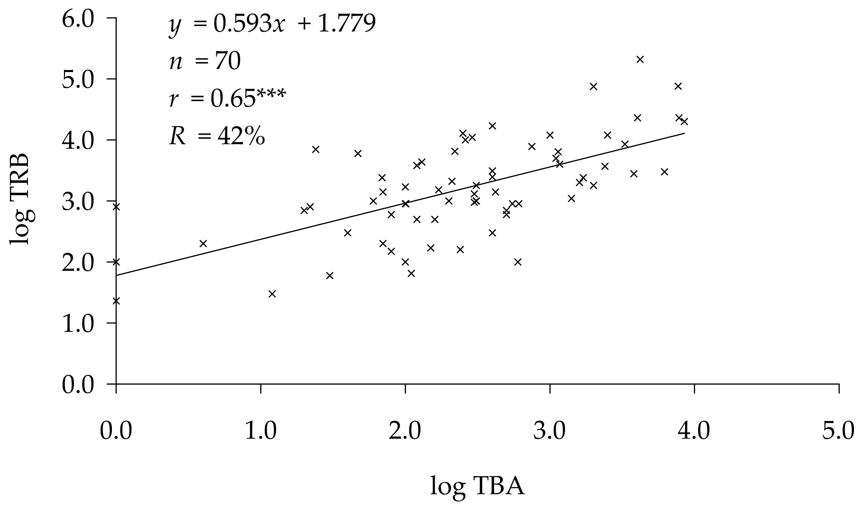

Figure 3. The relationship between the frequency of occurrence of bacilli (TBA) and thermoresistant bacteria (TRB) counts in feeds on feeding trough

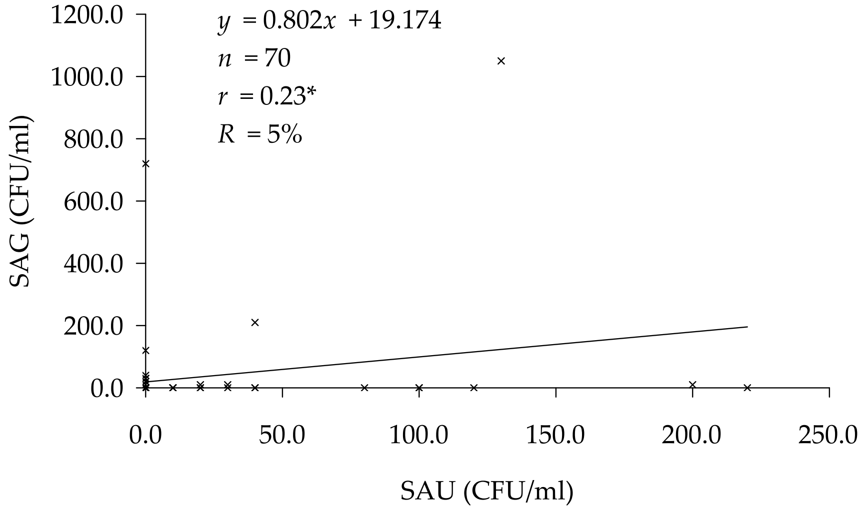

Figure 4. The relationship between the frequency of occurrence of main mastitis pathogens SAU and SAG in raw bulk cow milk

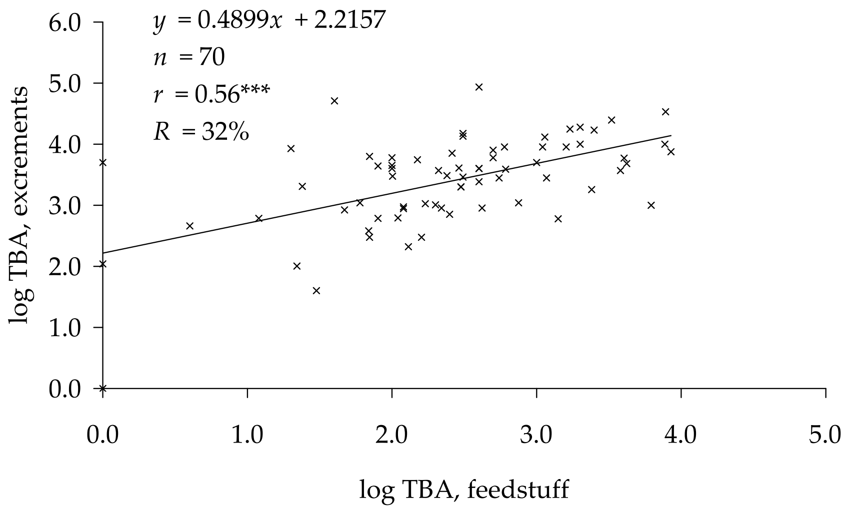

Figure 5. The relationship between the occurrence of bacilli (TBA) in feeds on feeding trough and in dairy cow excrements; SAG = *Streptococcus agalactiae*, SAU = *Staphylococcus aureus*

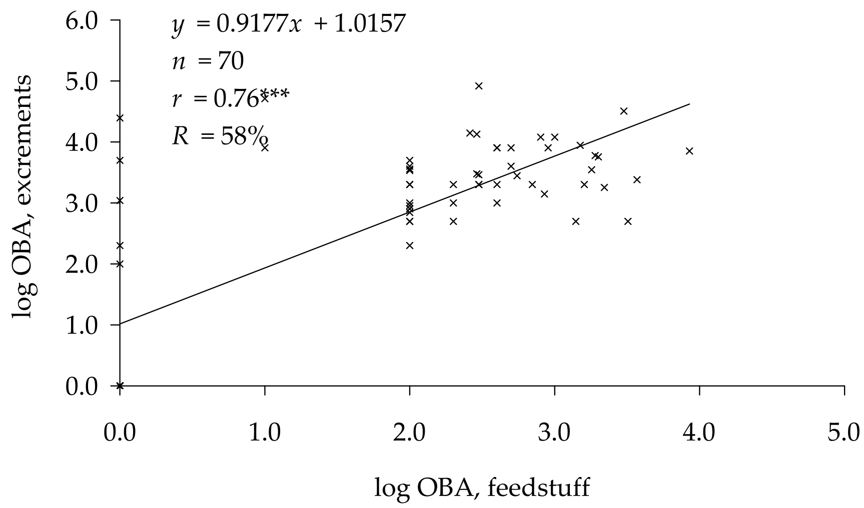

Figure 6. The relationship between the counts of other bacilli (OBA) in feeds on feeding trough and frequency of OBA occurrence in dairy cow excrements

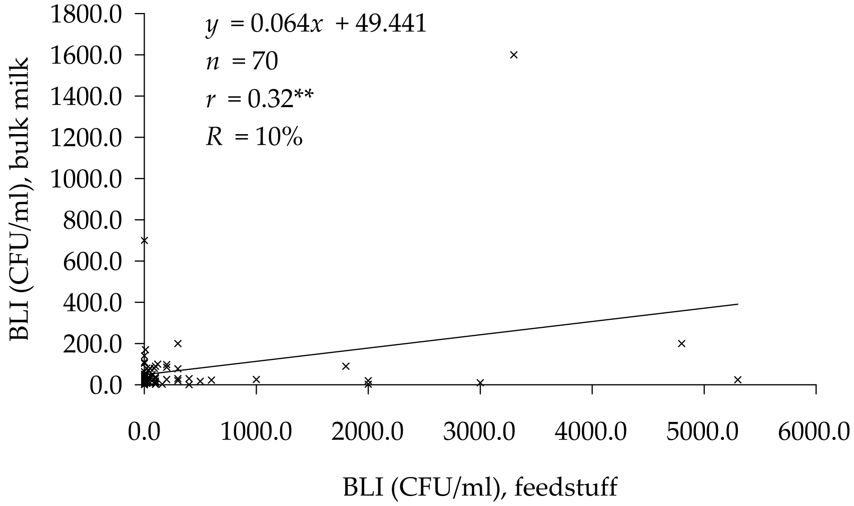

Figure 7. The relationship between *Bacillus licheniformis* (BLI) occurrence in feeds on feeding trough and in raw bulk cow milk

In total, the less narrow correlations were investigated in comparison to Petersen (1985) and Andersen and Jensen (1987 – cit. Kratochvíl, 1991).

Table 4. Significant correlations between hygienic parameters in different materials from dairy cow herds

Material	Parameter	Correlation coefficient
Excrements × milk	log TRB	0.24*
Feed × milk	BLI	0.32**
Feed × excrements	log TRB	0.38**
	log BLI	0.26*
	log OBA	0.76***
	log TBA	0.56***

$P < 0.05$; $P < 0.01$

Some correlation coefficients are in good agreement with the results of the paper published by Vyletělová (2001). It is possible to explain by generally very low milk contaminations by watching microorganisms as it was mentioned previously. It simultaneously gives evidence about the high technological hygiene of milk obtained in model dairy cow herds. Despite of this fact, proved dependences confirm the relationships between materials and possibilities of penetration of thermoresistant bacteria and bacilli through the technological chain along the axis feed – excrements – milk. The hygiene of harvest and preservation of rough fodders are therefore of basic importance for prevention of thermoresistant microorganisms in feeds, excrements of dairy cows and milk as well.

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ABSTRAKT

Technologicky obtížná, patogenní a potravinářsky riziková bakteriální kontaminace v syrovém mléce a dalších materiálech z chovu dojnic

Zpracování mléka na náročnější výrobky a bezpečnost mléčného potravinového řetězce vyžadují prevenci výskytu mikrororganismů, které mohou vytvářet spory. Je důležité provádět prevenci pomocí faremních hygienických opatření již při produkci syrového mléka. Uvedené mikroorganismy mohou následně ohrožovat kvalitu a bezpečnost mléčných potravin. Zmíněné předpokládá znalost faktorů, souvislostí a možností kontaminace mléka. Na sedmi farmách s dobrou úrovní mléčné užitkovosti, chovu a hygienických podmínek byl po tři roky v letním (S) a zimním (W) krmném období sledován výskyt hygienicky významných skupin mikroorganismů ve směsném krmivu (F) na žlabu, ve výkalech (E) a v bazénovém mléce (M). Soubor byl dobře vyvážen z hlediska dalších možných faremních faktorů (nadmořská výška, plemeno dojnic atd.). Celkem bylo odebráno 70 vzorků každého jmenovaného materiálu. Sledován byl počet termorezistentních mikroorganismů (TRB), počet bacilů celkem (TBA), četnost výskytu *Bacillus cereus* (BCE) a *Bacillus licheniformis* (BLI) a počet bacilů ostatních (OBA) ve všech jmenovaných materiálech a četnost výskytu hlavních patogenů mastitid *Streptococcus agalactiae* (SAG) a *Staphylococcus aureus* (SAU) v mléce. Průměrné výsledky zřetelně nepřekračovaly definované standardní limity, zejména pro mléko, které bylo vhodné ke spotřebě za syrova či k výrobě mléčných výrobků bez tepelného ošetření podle platné potravinářské legislativy EU. Variabilita hygienických parametrů byla poměrně vysoká: krmivo od 179 (TBA) do 315 % (TRB); výkaly od 178 (BLI) do 350 % (TRB); mléko od 117 (TBA) do 459 % (SAG). V letním období se zvyšovalo TRB v krmivech na žlabu ($P > 0{,}05$). Počty bacilů byly mezi letním a zimním obdobím vyrovnané. V letním období zvyšovalo BLI ve výkalech ($P \leq 0{,}05$), opačný trend byl pozorován u BCE. Sezona neovlivnila SAG a SAU v mléce, vyšší ($P \leq 0{,}01$) byly počty BCE v letním období, pro BLI byl trend opačný ($P \leq 0{,}05$). Vliv sezony na výskyt technologicky a zdravotně obtížných mikroorganismů v M, F a E dojnic nebyl celkově potvrzen. Mezi ukazateli byla provedena regresní analýza. V F byly významné pozitivní vztahy mezi bacily a TRB, podobně u E (korelační koeficienty od 0,32 do 0,65; $P \leq 0{,}01$). Vztahy mezi TRB a výskytem bacilů v M byly méně těsné (0,30; $P \leq 0{,}05$) než v F a E. Nevýznamné vztahy byly mezi TRB a výskytem hlavních mastitidních patogenů v mléce (SAG $r = -0{,}14$ a SAU $r = 0{,}11$; obojí $P > 0{,}05$). Dokládají účinnost pasterace při likvidaci těchto patogenů. Vztah SAU × SAG v M byl 0,23 ($P < 0{,}05$). Naznačuje souběžný vliv zdravotního stavu stáda na výskyt obou patogenů. Nejvíce významných vztahů (0,26 až 0,76; $P \leq 0{,}05$ až $P \leq 0{,}001$) bylo ve vazbě F × E uvnitř TRB, BLI, OBA a TBA. Změny v E byly v 7–58 % závislé na změnách v F. Výskyty TRB a BLI v M byly významně ovlivněny (0,24 a 0,32; $P \leq 0{,}05$ a $P \leq 0{,}01$) jejich výskyty ve E a F. Závislosti potvrzují vztahy mezi materiály a možnosti pronikání TRB a bacilů řetězcem technologie po ose krmivo – výkaly – mléko. Pro prevenci má hygiena sklizně a konzervace objemných krmiv zásadní význam.

Klíčová slova: dojnice; hygiena; objemné krmivo; výkaly; mléko; termorezistentní mikroorganismy; bacily celkem; *Bacillus cereus*; *Bacillus licheniformis*; ostatní bacily; potravní patogeny; mastitidní patogeny; *Streptococcus agalactiae*; *Staphylococcus aureus*; bezpečnost potravinového řetězce; prevence

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