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Diversity of Microscopic Fungi in the Raw Milk from Latvian Organic Farms

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Abstract. Raw milk is an ideal growth medium for microorganisms (including many fungal species), as such milk provides all necessary nutrients and conditions for their growth. The objective of the present study was to investigate the diversity of microscopic fungi in the milk obtained in Latvian organic farms depending on cow herd size and season. The study was carried out on 547 milk samples taken from 14 organic dairy farms from December 2011 to November 2012. The farms were divided into three groups according to herd size: small (S – 3–30 cows per shed), medium (M – 31–60 cows per shed), and large (L – 61–120 cows per shed). Milk samples from all four quarters in the same sampling tube were studied. Yeasts and moulds were found in 63.1% and 44.2% of samples, respectively. The identified mould strains belonged to 15 genera – most frequently to *Absidia, Aspergillus*, *Apophysomyces*, *Mucor*, *Penicillium*, and *Rhizopus spp.* The highest occurrence of yeasts and moulds was in L herds (73.6% and 50.9%, respectively). Also, milk samples from L herds had the greatest degree of yeast and mould contamination (5.3 and 2.8 log CFU mL^{-1}, respectively). It was found that the occurrence of yeasts was similar by seasons (54.9–68.9%), varying from 5.5 log CFU mL^{-1} in winter to 2.1–3.3 log CFU mL^{-1} in other seasons. The occurrence of moulds was the highest in spring (61.6%) and autumn (58.5%), but in winter and summer it was twice lower. The milk samples obtained in winter and summer contained more mould colonies (2.7 and 2.5 log CFU mL^{-1}, respectively) than those obtained in spring and autumn (1.8–1.3 log CFU mL^{-1}, respectively).
Key words: Yeasts, moulds, milk quality, season, herd size.

Introduction

Raw milk is generally considered an ideal growth medium for microorganisms, including many fungal species, because such milk provides all necessary nutrients and conditions for their growth (Callon *et al*., 2007; Delavenne *et al*., 2011; Spanamberg, Hartfelder, Meneghello Fuentefria, & Valente, 2004; Wouters, Ayad, Hugenholtz, & Smit, 2002). The occurrence of microscopic fungi in raw milk can take place during milking, handling, storage and other pre-processing activities; besides, it is influenced by the animal's physiological state and the weather and breeding conditions (Callon *et al*., 2007; Spanamberg, Hartfelder, Meneghello Fuentefria, & Valente, 2004; Vacheyrou *et al*., 2011).

Compared to bacterial diversity, only a few authors have studied the composition of fungal communities in raw milk, even though the knowledge of fungal contaminants in raw milk may be of interest for the maintenance of high hygienic practices. Besides, fungi have great importance in the technological processes of making dairy products and they can be used to judge milk sanitary quality and the conditions of dairy production (Cocolin, Aggio, Manzano, Cantoni, & Comi, 2002; Delavenne *et al*., 2011; Spanamberg, Hartfelder, Meneghello Fuentefria, & Valente, 2004).

The objective of the present study was to investigate the diversity of microscopic fungi in the milk obtained in Latvian organic farms depending on cow herd size and season. This is the first published article about microscopic fungi in the raw milk obtained in Latvia and a significant study about microbiological quality of the raw milk from Latvia's organic farms.

Materials and Methods

Five hundred and forty seven raw milk samples from 14 organic dairy farms, located in various parts of Latvia (regions of Kurzeme, Latgale, Vidzeme, and Zemgale), were collected in winter period (December 2011), when dairy cows were housed indoors, and in autumn period (October–November 2012), spring period (April–May 2012), and summer period

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(August 2012), when the cows were kept outdoors grazing on pastures. The organic farms are registered by the state control institutions. The size of the cow herds varied from 3 to 120 animals in a cow shed: four herds with 3–30 cows (S; number of milk samples n=86), four herds with 31–60 cows (M; n=250), and five herds with 61–120 cows (L; n=210) in a cow shed. Altogether, 144 samples were collected in winter, 138 samples – in spring, 159 samples – in summer, and 106 samples – in autumn. For sampling, 15 lactating cows were chosen from each herd. Milk samples in herds with less than 15 cows were collected from all lactating animals. The study included various breeds (Latvian Brown, Holstein, and Danish Red) as well as different varieties of cross breeds from the first to ninth lactation.

Milk samples from all four quarters in the same sampling tube were collected by trained farm personnel. The samples were collected in sterile 7-mL Vacutainers (*Vacutest Kima*, Italy), immediately transported to the Laboratory of Microbiology of the Research Institute of Biotechnology and Veterinary Medicine "Sigra" (Sigulda, Latvia) under the temperature of 10 °C, and then frozen at -20 °C for 2–6 weeks until analysis.

For the enumeration of yeasts and moulds, Sabouraud Dextrose agar (*Biolife Italiana*, Italy) was used according to the standard LVS ISO 21527-1:2008 "Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of yeasts and moulds – Part 1: Colony count technique in products with water activity greater than 0.95". Three dilutions in peptone water (*Biolife Italiana*, Italy) were prepared from the milk samples, then 1 mL per plate of the original sample and of each dilution was transferred into sterile plates. The plates were incubated at 22.5±1 °C for 10–14 days for identification. The identification of microscopic fungi belonging to genus was based on colony characteristics (pigmentation, shape, background colour) and on microscopic examination according to indications of authors (Carter & Wise, 2004; Hungerford, Campbell, & Smith, 1998).

For statistical analyses, the IBM SPSS Statistics version 21 was used. Descriptive statistics for the frequency of isolated microscopic fungi and average count of yeasts and moulds was done. Within season, herd type, count of yeasts and moulds, and group of moulds, bivariate analysis was performed to determine the strength of linear relationship between the variables; $p<0.05$ was considered statistically significant.

Results

Out of the total number of raw milk samples (n=547), yeasts were found in 345 samples (63.1%) and moulds were found in 242 samples (44.2%). Depending on herd size, the occurrence of yeasts was 66.3% in S herds, 51.1% in M herds, and 73.6% in L herds. The average number of microscopic fungi depending on herd size is presented in Fig. 1. The results showed that the greatest degree of yeast contamination was in L herds (210011 CFU mL^{-1} or 5.3 log CFU mL^{-1}),

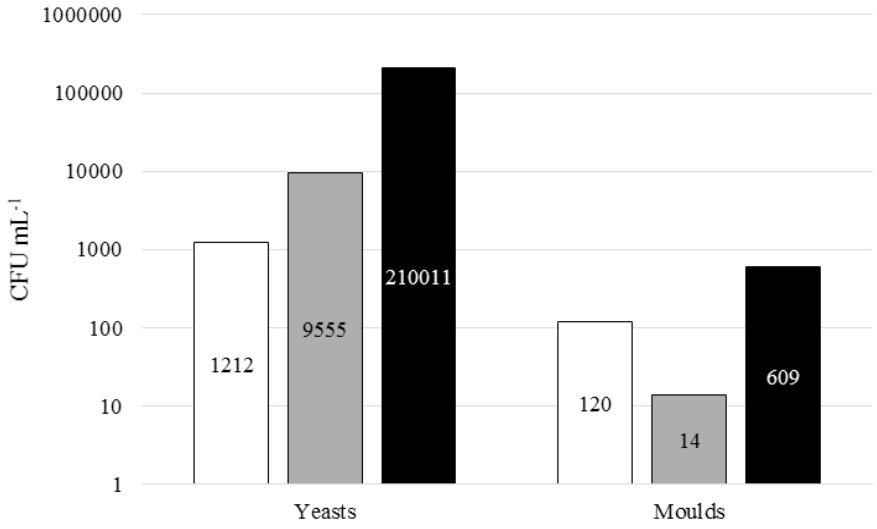


Fig. 1. The average number of yeasts and moulds depending on herd size:
□ – S (3–30 cows in a cowshed); ■ – M (31–60 cows in a cowshed); ■ – L (61–120 cows in a cowshed).

followed by M herds (9555 CFU mL^{-1} or 4.0 log CFU mL^{-1}), but the least contaminated samples were detected in S herds (1212 CFU mL^{-1} or 3.1 log CFU mL^{-1}). The occurrence of moulds was 26.7% in S herds, 45.0% in M herds, and 50.9% in L herds. The greatest degree of mould contamination was in L herds (609 CFU mL^{-1} or 2.8 log CFU mL^{-1}), then follow S herds (120 CFU mL^{-1} or 2.1 log CFU mL^{-1}), but the least contaminated samples were detected in M herds (14 CFU mL^{-1} or 1.1 log CFU mL^{-1}) (Fig. 1).

Depending on season, the occurrence of yeasts was similar in all samples (54.9–68.9%). The greatest number of raw milk samples contaminated with moulds was detected in spring (61.6%) and autumn (58.5%), whereas in winter and summer the moulds were isolated twice less frequently (about 31%).

The average number of microscopic fungi depending on season is presented in Fig. 2. The greatest degree of yeast contamination was in winter (321937 CFU mL^{-1} or 5.5 log CFU mL^{-1}), followed by summer (2224 CFU mL^{-1} or 3.3 log CFU mL^{-1}) and spring (583 CFU mL^{-1} or 2.8 log CFU mL^{-1}), but the least contaminated samples were detected in autumn (117 CFU mL^{-1} or 2.1 log CFU mL^{-1}). Mould isolation gave similar results: Milk samples obtained in winter (562 CFU mL^{-1} or 2.7 log CFU mL^{-1}) and summer (312 CFU mL^{-1} or 2.5 log CFU mL^{-1}) contained more mould colonies than those obtained in spring (64 CFU mL^{-1} or 1.8 log CFU mL^{-1}) and autumn (22 CFU mL^{-1} or 1.3 log CFU mL^{-1}).

Bivariate correlation analysis revealed that the effects of herd size on the yeast and mould count and isolated groups of moulds were significant ($p<0.05$) with a weak positive linear relationship (Table 1). The effect of season on yeast count was significant ($p<0.01$), but season's effect on mould count and isolated mould groups was not statistically significant.

The genera of isolated moulds are shown in Table 2. The mould genera most frequently isolated during all four seasons were *Aspergillus, Penicillium*, *Absidia*, and *Mucor spp.*: 44.2%, 28.9%, 16.1%, and 15.3%, respectively. Other more often distributed moulds belonged to *Rhizopus spp.* (5.0%) and *Apophysomyces spp.* (3.3%), which also were found throughout the year except for winter samples. The moulds isolated less frequently belonged to *Cladosporium spp.* (2.1%) and *Trichoderma spp.* (1.2%) and were detected in spring and autumn. Isolation frequency of other moulds did not exceed 1% of all examined raw milk samples, and among them were genera of *Chaetomium*, *Cunningchamellas*, *Geotrichum*, *Fusarium*, *Scedosporium*, *Scopulariopsis*, and *Sepedonium spp.*

The moulds of the genus *Trichoderma* were present in spring and autumn milk samples, but *Cladosporium spp.* were isolated in summer and autumn. The colonies that belonged to *Rhizopus* and *Apophysomyces spp.* were found in the milk samples taken in all seasons except for the samples taken in winter.

The presence of *Aspergillus spp.* in winter milk samples was found significantly less frequently (only 6.7%) compared to the samples collected in other seasons (44.2–66.0%). *Penicillium spp.* in the winter (6.7%) and summer (8.0%) milk samples were distributed less than in spring and autumn

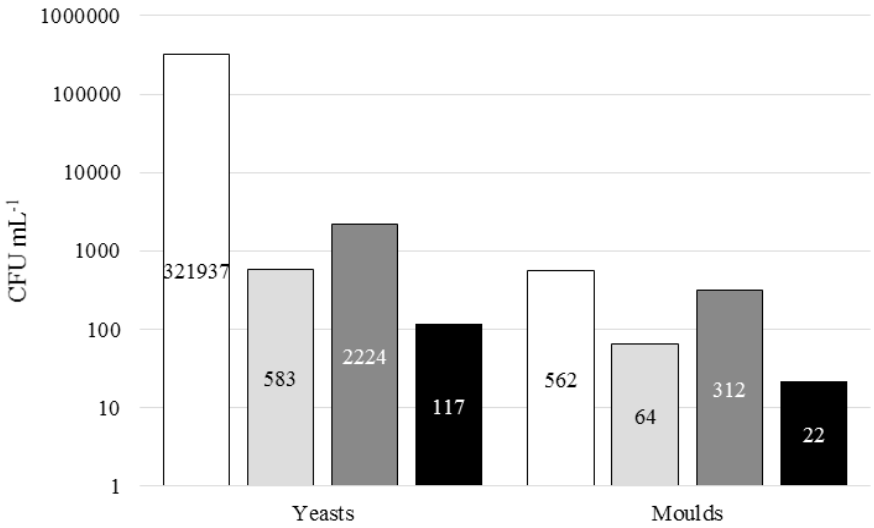


Fig. 2. The average number of yeasts and moulds depending on season:
□ – winter; ■ – spring; ■ – summer; ■ – autumn.

Table 1

Correlation among herd size, season, number of yeasts, number of moulds, and genus of moulds

		Herd size	Season	Number of yeasts	Number of moulds	Genus of moulds
Herd size	Pearson correlation	1	0.000	0.096*	0.121**	0.140*
	Significance (two-tailed)		1.000	0.024	0.005	0.029
	n	920	920	547	547	244
Season	Pearson correlation	0.000	1	-0.122**	-0.079	-0.113
	Significance (two-tailed)	1.000		0.004	0.066	0.079
	n	920	920	547	547	244
Number of yeasts	Pearson correlation	0.096*	-0.122**	1	0.003	0.045
	Significance (two-tailed)	0.024	0.004		0.945	0.488
	n	547	547	547	546	242
Number of moulds	Pearson correlation	0.121**	-0.079	0.003	1	-0.008
	Significance (two-tailed)	0.005	0.066	0.945		0.904
	n	547	547	546	547	242
Genus of moulds	Pearson correlation	0.140*	-0.113	0.045	-0.008	1
	Significance (two-tailed)	0.029	0.079	0.488	0.904	
	n	244	244	242	242	244

* – Correlation was significant at the 0.05 level (two-tailed).
** – Correlation was significant at the 0.01 level (two-tailed).

Table 2

The genera of moulds isolated from raw milk samples during a year

Genus of moulds	Season, and number of examined samples				
	Winter, n=144	Spring, n=138	Summer, n=159	Autumn, n=106	All seasons, n=547
	Number of samples containing moulds				
	n=45	n=85	n=50	n=62	n=242
	Percentage of samples containing moulds				
Aspergillus	6.7	47.1	66.0	50.0	44.2
Penicillium	6.7	52.9	8.0	29.0	28.9
Absidia	13.3	10.6	8.0	32.3	16.1
Mucor	20.0	15.3	14.0	12.9	15.3
Rzhizopus	0.0	3.5	10.0	6.5	5.0
Apophysomyces	0.0	2.4	6.0	4.8	3.3
Cladosporium	0.0	0.0	8.0	1.6	2.1
Trichoderma	0.0	1.2	0.0	3.2	1.2
Chaetomium	0.0	0.0	2.0	0.0	0.4
Cunningchamella	0.0	0.0	2.0	0.0	0.4
Fusarium	0.0	0.0	2.0	0.0	0.4
Geotrichum	2.2	0.0	0.0	0.0	0.4
Scedosporium	0.0	0.0	0.0	1.6	0.4
Scopulariopsis	0.0	1.2	0.0	0.0	0.4
Sepedonium	0.0	0.0	2.0	0.0	0.4
Unknown	51.1	1.2	0.0	0.0	9.9

(52.9–29.0%). *Absidia spp.* were isolated in autumn (32.3%) much more frequently than in other seasons (8.0–13.3%).

Discussion

The results of the present study showed that out of the total number of the raw milk samples (n=547) examined, the occurrence of yeasts and moulds made 63.1% (n=345) and 44.2% (n=242), respectively. Other researchers from Europe have reported a higher or similar distribution of fungi in raw milk. For example, Torkar and Vengus from Slovenia (2008) detected moulds in 63.3% of milk samples, and yeasts in 95.0% of milk samples, whereas Pesic-Mikulec, Stojanovic, and Jovanovic from Serbia (2005) found 57.0–100.0% of milk samples contaminated with microscopic fungi (including both yeasts and moulds).

In our research, depending on herd size, the highest occurrence of yeasts (73.6%) and the greatest degree of yeast contamination (5.3 log CFU mL^{-1}) were established in the milk samples collected from L herds; besides, these samples demonstrated also the highest occurrence of moulds (50.9%) and the greatest degree of mould contamination (2.8 log CFU mL^{-1}).

Yeasts and moulds in the raw milk samples from herds with a smaller number of cows were distributed less than in the largest herds: in S herds – 66.3% of samples with yeasts, and 26.7% of samples with moulds; and in M herds – 51.1% of samples with yeasts, and 45.0% of samples with moulds. Also, the milk samples taken from S and M herds contained less yeasts (3.1 log CFU mL^{-1} and 4.0 log CFU mL^{-1}, respectively) and moulds (2.1 log CFU mL^{-1} and 1.1 log CFU mL^{-1}, respectively) than the samples from L herds.

In the literature, there are few data about the relationship between the amount of microscopic fungi in milk and the herd size. It is hypothesized that differences in herd size and farm management practices may influence the microbial quality of milk (Cempirkova, 2006; Coorevits *et al*., 2008; Jayarao, Pillai, Sawant, Wolfgang, & Hegde, 2004). A study of Mallet *et al*. (2012) revealed that a greater degree of yeast contamination was detected in the milk obtained from herds with more than 60 cows compared to herds with less than 30 cows. This observation corresponds to our data – namely, that the highest occurrence of microscopic fungi was found in L herds, where herd size varied from 61 to 120 cows.

Many researchers guess that the level of microbial contamination of raw milk (fungal content is a part of the overall microbial composition) is influenced by dairy cow health and hygiene, by the hygiene of the environment where dairy cows are housed and milked, by the methods of udder preparation and milking technique, by the methods of cleaning and sanitation of milking machines and milk cisterns, by the speed of milk cooling to a required temperature, and by milk storage time (Cempirkova, 2006; Coorevits *et al*., 2008; Jayarao, Pillai, Sawant, Wolfgang, & Hegde, 2004; Mallet *et al*., 2012; Vacheyrou *et al*., 2011). Our research revealed that these conditions are met more successfully in the farms with a moderately low herd size – 31–60 cows per shed. The farms where herd size is small (less than 31 in our study) usually are not so well equipped with high-tech milking machines and cooling facilities, producing milk of a lower microbial quality (Dankow, Wojtowsk, & Fahr, 2004), whereas large herds often have inferior hygienic conditions than those of a medium size because of the high animal density, especially in free stalls.

Depending on season, the highest occurrence of moulds was observed in spring (61.3%) and autumn (58.5%), but in the winter and summer milk samples it was twice lower (about 31%); however, the mean mould colony count per mL in the winter (2.7 log CFU mL^{-1}) and summer milk samples (2.5 log CFU mL^{-1}) was higher than in the milk samples obtained in other seasons.

The occurrence of yeasts was almost constant throughout the year (54.9–68.9%). The greatest degree of yeast contamination was in winter milk samples (5.5 log CFU mL^{-1}), followed by summer (3.3 log CFU mL^{-1}) and spring (2.8 log CFU mL^{-1}) milk samples, but the least contaminated samples were detected in autumn (2.1 log CFU mL^{-1}).

A study of the D. Pesic-Mikulec group revealed a significant correlation between the season and the count of moulds in raw milk (Pesic-Mikulec, Stojanovic, & Jovanovic, 2005). These authors found that the percentage of moulds in raw milk samples varied depending on geographical location and season of a year, i.e., from 46.64% in winter to 91.30% in autumn in the milk samples obtained from a region of alluvial plains by a river. Other researchers (Elmoslemany *et al*., 2010) had assumed that a warmer air temperature might allow the microorganisms to grow faster; however, their research demonstrated that variations in seasonal data could be influenced not only by air temperature and precipitation but also

by some management practices such as environment and cow hygiene.

In our study, the highest degree of yeast and mould contamination was found in winter milk samples, followed by summer milk samples. Many authors have reported on a higher number of yeasts and moulds in ensiled feed that was used mostly in winter season. These microorganisms very often transfer from feed to milk (Torkar & Teger, 2008). In view of these assumptions, it is possible that a warm ambient temperature in summer and a highly contaminated feed in winter favour a higher microscopic fungi concentration in raw milk compared to spring and autumn; however, this does not explain why just in autumn and spring the occurrence of microscopic fungi is distinctly greater.

In our study, the most common genera in the raw milk from all cow herds were *Aspergillus*, *Penicillium*, *Absidia*, *Mucor*, *Rhizopus*, and *Apophysomyces spp.* Except for *Apophysomyces spp.*, this coincides with the results of other studies on fungal diversity in a raw cow milk, carried out by Callon *et al*. (2006), Cocolin, Aggio, Manzano, Cantoni, and Comi (2002), Delavenne *et al*. (2011), Elmoslemany *et al*. (2010), Ministry of Education and Science of the Republic of Latvia (2010), Pesic-Mikulec, Stojanovic, and Jovanovic (2005), Roussel *et al*. (2011), Torkar and Vengus (2008), and Vacheyrou *et al*. (2011). In our study, *Fusarium spp., Cladosporium spp., Trichoderma spp., Scopulariopsis spp.,* and *Scedosporium spp.* were isolated more rarely, which also agrees with the findings of the above-mentioned authors. Besides, from some samples, we isolated moulds that have not been mentioned previously in the literature in connection with cow milk – *Chaetomium spp., Rhizomucor spp.*, and *Sepedonium spp*.

Yeasts themselves are not commonly the cause of spoilage of dairy products, unless they ferment lactose. In this case, they can grow rapidly and produce a characteristic yeasty flavour and obvious gas (Torkar & Teger, 2008). Moulds, like yeasts, are normally regarded as spoilage organisms in milk. Moulds are important in the milk that is used for the manufacture of cheese and other dairy products. Specified mould species called starter-moulds are used during a cheese manufacturing process with the aim to enhance the flavour and aroma of cheese and to modify its body and structure (Wouters, Ayad, Hugenholtz, & Smit, 2001). There is a study that suggests that non-starter yeasts and moulds can transfer from raw milk to raw milk cheese and may negatively influence cheese ripening (Lavoie, Touchette, St-Gelais, & Labrie, 2012). Although moulds are not expected to survive the pasteurization and sterilization treatments applied during milk processing, their presence in raw milk is undesirable because their enzymatic activities may alter milk constituents and affect organoleptic characteristics of dairy products, produce mycotoxins, and represent a potential health risk (Spanamberg, Hartfelder, Meneghello-Fuentefria, & Valente, 2004; Torkar & Teger, 2008; Torkar & Vengus, 2008). In rare cases, yeasts and moulds can cause mastitis in cows, especially after a prolonged use of antibiotics (Britt, 1998).

Several authors have stated that animal feeding stuffs are the source of the microscopic fungi of the barn environment. The studies on microbial transfers from the stable to the milk reveal that most of the fungal species found in milk were also found in the cow-shed and the milking parlor environments, indicating a large microbial transfer from shed to milking parlor and then to milk (Ministry of Education …, 2010; Torkar & Teger, 2008; Vacheyrou *et al*., 2011).

Our results showed that the most widely spread fungal genera found in raw milk are filamentous fungi (including *Penicillium spp*., *Aspergillus spp*., *Absidia spp*., *Rhizopus spp*., *Fusarium spp*., *Apophysomyces spp*., *Trichoderma spp*., *Sepedonium spp*., and *Scopulariopsis spp.*), which frequently occur in feed, in decaying vegetation, in soil, or in animal faeces. The other part of fungi belong to the dematiaceous filamentous fungi (including *Scedosporium spp*., *Chaetomium spp.,* and *Cladosporium spp*.) of the same location. Several of moulds, including *Absidia spp*., *Aspergillus spp*., and *Penicillium spp*., are isolated from foods and indoor air environment; they often cause food spoilage (Doctor Fungus, 2007).

Conclusions

1. Out of the total number of raw milk samples (547), yeasts and moulds were found in 345 (63.1%) and 242 (44.2%) samples, respectively.
2. The occurrence of yeasts in raw milk samples depending on the cow herd size made 66.3% in small herds, 51.1% in medium herds, and 73.6% in large herds. The highest degree of yeast contamination was in large herds (5.3 log CFU mL^{-1}), followed by medium herds (4.0 log CFU mL^{-1}), but the least contaminated samples were detected in small herds (3.1 log CFU mL^{-1}).

3. The occurrence of moulds in raw milk samples depending on the cow herd size was 26.7% in small herds, 45.0% in medium herds, and 50.9% in large herds. The highest degree of mould contamination was in large herds (2.8 log CFU mL^{-1}), followed by small herds (2.1 log CFU mL^{-1}), but the least contaminated samples were found in medium cow herds (1.1 log CFU mL^{-1}).
4. The occurrence of yeasts in raw milk samples depending on season was similar throughout the year and varied between 54.9% and 68.9%. The highest degree of yeast contamination was detected in winter (5.5 log CFU mL^{-1}), less – in summer (3.3 log CFU mL^{-1}) and spring (2.8 log CFU mL^{-1}), but the least – in autumn (2.1 log CFU mL^{-1}).
5. The occurrence of moulds in raw milk samples depending on season was the highest in spring (61.6%) and autumn (58.5%), whereas in winter and summer the moulds were isolated twice less frequently (about 31%). The raw milk samples in winter (2.7 log CFU mL^{-1}) and summer (2.5 log CFU mL^{-1}) contained more mould colonies per mL than in spring (1.8 log CFU mL^{-1}) and autumn (1.3 log CFU mL^{-1}).
6. The mould strains identified in raw milk samples belonged to 15 genera, of which *Aspergillus* (44.2%), *Penicillium* (28.9%), *Absidia* (16.1%), and *Mucor spp*. (15.3%) were detected most frequently – in all herds and during the whole year.
7. Bivariate correlation analysis revealed that the effects of herd size on the yeast and mould count and on isolated moulds were significant ($p<0.05$) with a weak positive linear relationship. The effect of season on yeast count was significant ($p<0.01$), while season's effect on mould count and isolated moulds was not statistically significant.

References

1. Britt, J.S. (1998). Mastitis in large animals. In Cynthia M. Kahn (Ed.), *Merck Veterinary Mannual* (8th ed.) (pp. 1012-1013). New York, USA: Merck & Co, Whitehouse station.
2. Callon, C., Duthoit, F., Delbes, C., Ferrand, M., Le Frileux, Y., De Cremoux, R., & Montel, M.C. (2007). Stability of microbial communities in goat milk during a lactation year: molecular approaches. *Systematic and Applied Microbiology*, 30, 547–560. DOI: 10.1016/j.syapm.2007.05.004.
3. Carter, G.R., & Wise, D.J. (2004). *Essentials of Veterinary Bacteriology and Mycology* (6th ed.). Iowa, USA: Iowa State Press.
4. Cempirkova, R. (2006). Factors negatively influencing microbial contamination of milk. *Agricultura tropica et subtropica,* 39(4). Retrieved from EZB database on the World Wide Web: http://www.agriculturaits.czu.cz.
5. Cocolin, L., Aggio, D., Manzano, M., Cantoni, C., & Comi, G. (2002). An application of PCR-DGGE analysis to profile the yeast populations in raw milk. *International Dairy Journal*, 12, 407–411. DOI: 10.1016/S0958-6946(02)00023-7.
6. Coorevits, A., De Jonghec, V., Vandroemmed, J., Reekmansa, R., Heyrmana, J., Messensc, W., … Heyndrickxc, M. (2008). Comparative analysis of the diversity of aerobic spore-forming bacteria in raw milk from organic and conventional dairy farms. *Systematic and Applied Microbiology*, 31(2), 126–140. DOI: 10.1016/j.syapm.2008.03.002.
7. Dankow, R., Wojtowsk, J., & Fahr, R.D. (2004). Hygienic quality of raw milk in relation to methods of production and storage. *Medycyna Weterynaryjna*, 60(1), 46–49.
8. Delavenne, E., Mounier, J., Asmani, K., Jany, J.L., Barbier, G., & Le Blay, G. (2011). Fungal diversity in cow, goat and ewe milk. *International Journal of Food Microbiology*, 151, 247–251. DOI: 10.1016/j.ijfoodmicro.2011.08.029.
9. Doctor Fungus. (2007, January). Descriptions of fungi. Retrieved from http://www.doctorfungus.org/
10. Elmoslemany, A.M., Keefe, G.P., Dohoo, I.R., Wichtel, J.J., Stryhn, H., & Dingwell, R.T. (2010). The association between bulk tank milk analysis for raw milk quality and on-farm management practices. *Preventive Veterinary Medicine*, 95(1–2), 32–40. DOI: 10.1016/j.prevetmed.2010.03.007.
11. Hungerford, L.L., Campbell, C.L., & Smith, A.R. (1998). *Veterinary mycology laboratory manual*. Iowa, USA: Iowa State Press.
12. Jayarao, B.M., Pillai, S.R., Sawant, A.A., Wolfgang, D.R., & Hegde, N.V. (2004). Guidelines for monitoring bulk tank milk somatic

cell and bacterial counts. *Journal of Dairy Science*, 87(10), 3561–3573. DOI: 10.3168/jds.S0022-0302(04)73493-1.

13. Lavoie, K., Touchette, M., St-Gelais, D., & Labrie, S. (2012). Characterization of the fungal microflora in raw milk and specialty cheeses of the province of Quebec. *Dairy Science and Technology*, 92(5), 455–468. DOI 10.1007/s13594-011-0051-4.
14. Mallet, A., Gueguen, M., Kauffmann, F., Chesneau, C., Sesboue, A., & Desmasures, N. (2012). Quantitative and qualitative microbial analysis of raw milk reveals substantial diversity influenced by herd management practices. *International Dairy Journal*, 27, 13–21. DOI: 10.1016/j.idairyj.2012.07.009.
15. Ministry of Education and Science of the Republic of Latvia. (2010, December). *Piena mikrobiālās piesārņošanas risku izvērtējums saistībā ar patērētāja veselībai drošas produkcijas ieguvi (Assesment of risks of milk microbial contamination in association with gaining of safe production for the consumer's health)*. Retrieved from http://izm.izm.gov.lv/upload_file/Zinatne/TOP/TOP08-02.pdf
16. Pesic-Mikulec, D., Stojanovic, L., & Jovanovic, L. (2005). Moulds associated with milk depending on climate and location. *Applied Ecology and Environmental Research*, 3(2), 61–65. Retrieved on the World Wide Web: http://www.ecology.kee.hu.
17. Roussel, S., Sudre, B., Reboux, G., Waser, M., Buchele, G., Vacheyrou, M., … Piarroux, R. (2011). Exposure to moulds and actinomycetes in Alpine farms: A nested environmental study of the PASTURE cohorts. *Environmental Research*, 111, 744–750. DOI: 10.1016/j.envres.2011.05.002.
18. Spanamberg, A., Hartfelder, C., Meneghello-Fuentefria, A., & Valente, P. (2004). Diversity and enzyme production by yeasts isolated from raw milk in Southern Brazil. *Acta Scientiae Veterinariae*, 32(3), 195–199. Retrieved from LUME database on the World Wide Web: http://hdl.handle.net/10183/20193. ISSN 1678-0345
19. Torkar, K.G., & Teger, S.G. (2008). The microbiological quality of raw milk after introducing the two-day's milk collecting system. *Acta agriculturae Slovenica,* 92, 61–74.
20. Torkar, K.G., & Vengus, A. (2008). The presence of yeasts, moulds and aflatoxin M1 in raw milk and cheese in Slovenia. *Food Control*, 19, 570–577. DOI: 10.1016/j.foodcont.2007.06.008.
21. Vacheyrou, M., Normand, A.C., Guyot, P., Cassagne, C., Piarroux, R., & Bouton, Y. (2011). Cultivable microbial communities in raw cow milk and potential transfers from stables of sixteen French farms. *International Journal of Food Microbiology*, 146, 253–262. DOI: 10.1016/j.ijfoodmicro.2011.02.033.
22. Wouters, J.T.M., Ayad, E.H.E., Hugenholtz, J., & Smit, G. (2002). Microbes from raw milk for fermented dairy products. *International Dairy Journal*, 12, 91–109. DOI: 10.1016/S0958-6946(01)00151-0.