

## Microbiological Quality of Raw Milk in the Czech Republic

KATEŘINA BOGDANOVIČOVÁ<sup>1</sup>, MARCELA VYLETĚLOVÁ-KLIMEŠOVÁ<sup>2</sup>, VLADIMÍR BABÁK<sup>3</sup>,  
LIBOR KALHOTKA<sup>4</sup>, IVANA KOLÁČKOVÁ<sup>3</sup> and RENÁTA KARPÍŠKOVÁ<sup>3</sup>

<sup>1</sup>Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic; <sup>2</sup>Dairy Research Institute, Prague, Czech Republic;

<sup>3</sup>Veterinary Research Institute, Brno, Czech Republic; <sup>4</sup>Faculty of Horticulture, Mendel University in Brno, Brno, Czech Republic

### Abstract

BOGDANOVIČOVÁ K., VYLETĚLOVÁ-KLIMEŠOVÁ M., BABÁK V., KALHOTKA L., KOLÁČKOVÁ I., KARPÍŠKOVÁ R. (2016): Microbiological quality of raw milk in the Czech Republic. Czech J. Food Sci., 34: 189–196.

The microbiological and hygienic quality of cow's, goat's and sheep's milk in the Czech Republic was evaluated. Milk (230 samples) was collected on 41 farms and investigated from May 2012 to October 2014. Milk was analysed for the presence of selected groups and types of bacteria: mesophilic microorganisms (total plate count – TPC), enterococci, *Enterobacteriaceae*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., *Campylobacter* spp., and *Listeria monocytogenes*. Besides these indicators and pathogenic agents, somatic cell count was determined as one of the indicators of mammary gland health in cows. TPC ranged between  $8.3 \times 10^2$  and  $1.2 \times 10^9$  CFU/ml and somatic cells between  $1.6 \times 10^4$  and  $6.8 \times 10^6$  cells/ml. The presence of *E. coli* was confirmed in 86.3% of samples and the colony counts ranged from  $1.0 \times 10^1$  to  $4.0 \times 10^6$  CFU/ml. The presence of verotoxigenic *E. coli* was confirmed in 3 samples (1.3%) (cow's milk 0%; goat's milk 6.3%; sheep's milk 4.4%). The presence of *S. aureus* was confirmed in 29.1% of the samples (cow's milk 26.9%; goat's milk 34.4%; sheep's milk 39.1%), but the numbers were very low ( $< 5.0 \times 10^2$  CFU/ml). *L. monocytogenes* was confirmed in 3 examined samples (1.3%) (cow's milk 0.6%; goat's milk 3.1%; sheep's milk 4.4%). *Salmonella* spp. and *Campylobacter* spp. were not detected in any of the samples tested.

**Keywords:** raw milk; TPC; SCC; enterococci; *Enterobacteriaceae*; *Escherichia coli*; *Staphylococcus aureus*; *Salmonella* spp.; *Campylobacter* spp.; *Listeria monocytogenes*

In the European Union, raw milk has received considerable attention. Hygienic limits for raw milk of cows and milk of other mammals have been established within the European Union (Regulation (EC) No. 853/2004). Values of the measured parameters indicate the health status of the mammary gland of mammals and are always assessed in raw milk. A failure to comply with these parameters creates a serious risk to consumer health because microbiologically contaminated raw milk can become a source of serious foodborne illnesses. The study of OLIVER *et al.* (2009) from the United States in 2002–2008 revealed 12 confirmed outbreaks of illnesses associated with the consumption of raw milk that had been caused by

*Listeria monocytogenes* (1 outbreak), *Campylobacter* spp. (5 outbreaks), *Salmonella* spp. (4 outbreaks), and verotoxigenic *Escherichia coli* (2 outbreaks). According to the report by the European Food Safety Authority (EFSA) in 2010 (EFSA 2012), *Campylobacter* spp. was found in raw milk samples in four EU countries (Germany, Italy, Hungary, and Slovakia). In the Czech Republic, 219 samples of raw milk from 15 farms taken from 27 milk vending machines were analysed in 2010. Considerable variations in the microbiological quality were observed between the farms, with repeated detections of pathogens on some farms. *Staphylococcus aureus* was detected in 124 samples (56.6%). *Campylobacters*, mostly

Supported by Ministry of Agriculture of the Czech Republic, Projects Nos QJ1230044, QJ1210284, and MEYS NPU program LO1218.

*Campylobacter jejuni*, were detected in 10 samples (4.6%), *Listeria monocytogenes* in 4 samples (1.8%), and *Salmonella* spp. in 7 samples (3.2%). The following serotypes were detected: *S. Typhimurium* DT104, *S. Enteritidis* PT13a, *S. Bovismorbificans*, and *S. Infantis* (KARPIŠKOVÁ *et al.* 2011).

Limits for somatic cell count (SCC) and total plate count (mesophilic microorganisms) – TPC in cow's raw milk are given by Regulation (EC) No. 853/2004 of the European Parliament and of the Council, which lays down the count of microorganisms at  $30^{\circ}\text{C} \leq 10^5$  CFU/ml and SCC at  $\leq 4 \times 10^5$ /ml. For raw milk from other species, the limit for the TPC of microorganisms at  $30^{\circ}\text{C}$  is  $\leq 1.5 \times 10^6$  CFU/ml.

The aim of the present study was to investigate the microbiological quality and safety of cow's, goat's, and sheep's milk produced in the Czech Republic, to evaluate the occurrence of selected groups and species of bacteria and to compare the results with legislative parameters if they are established by European or national legislation, to evaluate the relationship between CPM and somatic cells, and monitor the probability of *S. aureus* occurrence relative to SCC in cow's milk.

## MATERIAL AND METHODS

Sampling was conducted at irregular intervals from May 2012 to October 2014 on 41 farms in the Czech Republic. Samples of cow's (175), goat's (32), and sheep's (23) milk (250 ml) were collected into sterile containers and transported to the laboratory in insulated containers at  $4 \pm 1^{\circ}\text{C}$ . Upon delivery to the laboratory, samples were immediately processed and analysed. The farms were monitored for the hygienic quality of raw milk TPC, counts of enterococci, *Enterobacteriaceae*, *Escherichia coli*, as well as *Staphylococcus aureus*, and the presence of *Listeria monocytogenes*, *Campylobacter* spp., and *Salmonella* spp. Somatic cell count was also monitored as one of the health indicators of the mammary gland.

Laboratory tests were conducted in accordance with valid Czech standards (ČSN). To determine the somatic cell count, the fluoro-opto-electronic method (Fossomatic 90) was used according to SOP 32 and the device Delaval cell counter DCC (MIKROS-tech, Tumba, Sweden). TPC values were determined according to the standard ČSN EN ISO 4833:1991, GTK culture medium (HiMedia, Mubai, India) was used for culture, and incubation was carried out at  $30^{\circ}\text{C}$  for 72 hours.

The determination of bacteria from the family *Enterobacteriaceae* was performed according to ČSN EN ISO 21528-1:2004 with inoculation onto selective VRBL agar medium (HiMedia, India). The suspect colonies were tested for (negative) oxidase reaction (OXItest; Erba-Lachema, Brno, Czech Republic) and glucose fermentation.

The number of enterococci was determined by inoculating 0.2 ml of the sample suspension onto the surface of selective Slanetz-Bartley agar (HiMedia, India). Incubation was carried out aerobically at  $37^{\circ}\text{C}$  for 24–48 hours.

The enumeration of *E. coli* was performed according to the method defined by ČSN EN ISO 16649-1:2003, known as Horizontal method for the determination of  $\beta$ -glucuronidase-positive *Escherichia coli*, by the technique of counting colonies cultured at  $44^{\circ}\text{C}$ , using 5-bromo-4-chloro-3-indolyl- $\beta$ -D-glucuronide. The detection was performed by a modification of the ČSN ISO 16649-2:2003 method after sample enrichment in buffered peptone water (Oxoid, Hampshire, UK) at  $37^{\circ}\text{C}$  for 24 h with subsequent culture on TBX agar ( $44^{\circ}\text{C}$ , 24 h). Confirmation of suspect isolates consisted in negative oxidase reaction (OXItest; Erba-Lachema, Czech Republic) and in positive indole reaction (COLItest; Erba-Lachema, Czech Republic). In *E. coli* strains, the presence of selected virulence factors was monitored. For the detection of genes encoding selected virulence factors *eaeA* (intimin), *hly* (hemolysin), *stx*<sub>1</sub>, and *stx*<sub>2</sub> (verotoxin 1 and 2), a multiplex PCR was used according to FAGAN *et al.* (1999).

Enumeration of coagulase-positive staphylococci was performed according to ČSN EN ISO 6888-1:1999. The detection was carried out after propagation in peptone water (Oxoid, UK). Baird-Parker Medium (Oxoid, UK) was used for the culture. The identification of suspect colonies was performed by the detection of coagulase (Denka Seiken, Tokyo, Japan). Confirmation of suspect strains of *S. aureus* was performed by a polymerase chain reaction with the specific SA442 fragment detection (MARTINEAU *et al.* 1998).

Detection of *Salmonella* spp. was performed according to ČSN EN ISO 6579:2003. After enrichment in peptone water, selective enrichment in RVS and MKTTN media (both Oxoid, UK) was carried out. This was followed by inoculation onto RAMBACH (Merck, Darmstadt, Germany) and XLD (Oxoid, UK) agars.

The presence of *Campylobacter* spp. was monitored according to ČSN EN ISO 10272-1:2003. Enrichment was carried out in Bolton Broth with horse blood and

doi: 10.17221/25/2016-CJFS

subsequent inoculation onto CCDA medium (both Oxoid, UK). The incubation was carried out under microaerophilic conditions at 42°C for 48 hours.

The presence of *Listeria monocytogenes* was determined according to ČSN EN ISO 11290-1:1999, primary enrichment took place in Half Fraser Broth at 30°C for 24 h, and it was followed by inoculation onto Full Fraser Broth (both Oxoid, UK) at 37°C for 24–48 hours. Inoculation was performed onto ALOA Agar (BioRad, Steenvoorde, France) and culture was carried out at 37°C for 24–48 hours.

The statistical analysis was performed using programs GraphPadPrism 5.04 (GraphPad, Inc., San Diego, USA) and Statistica 12.0 (Dell, Inc., Tulsa, USA).

## RESULTS AND DISCUSSION

The EFSA summary report on trends and sources of zoonoses, zoonotic agents, and food-borne outbreaks (2012) showed that from 1982 to 2010, 64 cases of dairy-associated infections were reported in Europe, in the United States, and Canada (VERRAES *et al.* 2015). Based on this information, the following study aimed at monitoring of somatic cell count and selected microbiological indicators of raw milk was conducted.

The results of somatic cell count determination indicate that the average values of SCC statistically significantly depend on the origin of milk ( $P < 0.01$ , Kruskal-Wallis test). Figure 1 shows the detected SCC

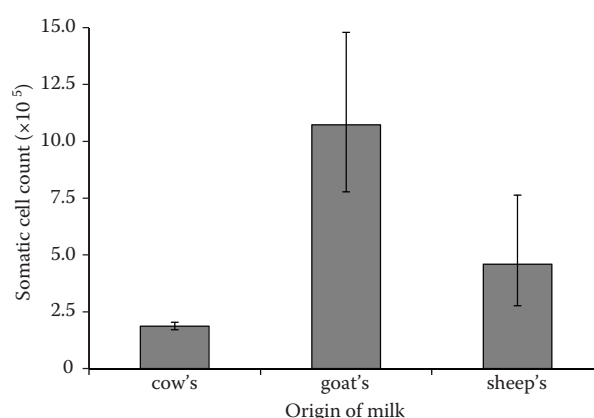


Figure 1. Somatic cell counts (SCC) in the investigated milk samples of different origin

The columns represent geometric means of SCC, vertical bars correspond to 95% confidence intervals of the geometric means

values: the lowest in cow's milk ( $1.6 \times 10^4$  to  $9.9 \times 10^5$ /ml) and the highest in goat's milk ( $1.7 \times 10^5$  to  $6.8 \times 10^6$ /ml). A statistically significant difference in SCC was demonstrated between cow's milk and milk of the other species, and also between goat's and sheep's milk. The number of somatic cells in small ruminants' milk is not commonly measured, and therefore, no mandatory limits exist. Some authors, however, believe that whereas SCC counts from  $2.5 \times 10^2$  to  $3.0 \times 10^5$ /ml in dairy cows are considered as threshold values between infected

Table 1. Descriptive statistics for somatic cells counts (SCC) and total plate count (TPC) according to milk origin

Statistics	Somatic cells count (ml)			Total plate count of microorganisms (ml)		
	cow's (1)	goat's (2)	sheep's (3)	cow's (1)	goat's (2)	sheep's (3)
<i>n</i>	149	29	24	154	30	21
Minimum	$1.6 \times 10^4$	$1.7 \times 10^5$	$5.5 \times 10^4$	$1.0 \times 10^3$	$9.3 \times 10^2$	$8.3 \times 10^2$
Maximum	$9.9 \times 10^5$	$6.8 \times 10^6$	$1.9 \times 10^6$	$3.0 \times 10^7$	$1.2 \times 10^9$	$5.9 \times 10^6$
Geometric mean	$1.9 \times 10^5$	$1.1 \times 10^6$	$4.6 \times 10^5$	$2.5 \times 10^4$	$5.7 \times 10^5$	$6.0 \times 10^5$
95% confidence interval	$1.7 \times 10^5$	$7.8 \times 10^5$	$2.8 \times 10^5$	$1.8 \times 10^4$	$1.9 \times 10^5$	$2.0 \times 10^5$
Of geometric mean	$2.0 \times 10^5$	$1.5 \times 10^6$	$7.6 \times 10^5$	$3.4 \times 10^4$	$1.7 \times 10^6$	$1.8 \times 10^6$
Median	$2.0 \times 10^5$	$1.1 \times 10^6$	$6.8 \times 10^5$	$2.0 \times 10^4$	$7.4 \times 10^5$	$1.1 \times 10^6$
Lower quartile	$1.3 \times 10^5$	$6.7 \times 10^5$	$2.3 \times 10^5$	$7.0 \times 10^3$	$1.3 \times 10^5$	$2.3 \times 10^5$
Upper quartile	$2.7 \times 10^5$	$1.5 \times 10^6$	$1.5 \times 10^6$	$8.1 \times 10^4$	$2.2 \times 10^6$	$3.9 \times 10^6$
Interquartile range	$1.3 \times 10^5$	$8.0 \times 10^5$	$1.3 \times 10^6$	$7.4 \times 10^4$	$2.1 \times 10^6$	$3.7 \times 10^6$
Kruskal-Wallis test		$P < 0.01$			$P < 0.01$	
Dunn's post-hoc tests	(1) : (2)	$P < 0.01$		(1) : (2)	$P < 0.01$	
	(1) : (3)	$P < 0.01$		(1) : (3)	$P < 0.01$	
	(2) : (3)	$P < 0.05$		(2) : (3)	$P > 0.05$	

*n* – No. of samples

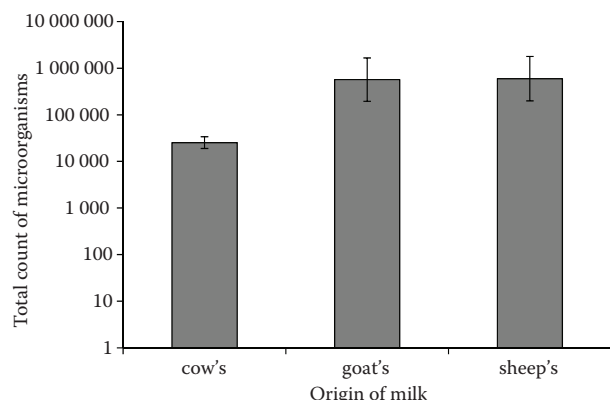


Figure 2. Total plate count (TPC) of microorganisms in the investigated milk samples of different origin

The columns represent geometric means of TPC, vertical bars correspond to 95% confidence intervals of the geometric means

and non-infected mammary gland, this cannot be stated unequivocally in sheep and goats (FTHENAKIS *et al.* 1991; GONZÁLEZ-RODRÍGUEZ *et al.* 1995). The above-mentioned authors also reported that healthy sheep tend to have higher SCC values than healthy cows. BUFANO *et al.* (1996) documented that higher SCC values ( $> 1 \times 10^6$ /ml) are commonly found in the milk of healthy sheep, goats, and cows.

Regulation (EC) No. 853/2004 of the European Parliament lays down specific hygiene limits for cow's raw milk which should not be exceeded. The values of CPM  $1.0 \times 10^5$  CFU/ml and  $1.5 \times 10^6$  CFU/ml should not be exceeded for cow's raw milk and for raw milk from species other than cows, respectively. Both values are measured in raw milk before processing, and their values indicate the health status of the mammary gland. A failure to comply with hygiene requirements creates a potential risk to consumers' health, as microbiologically contaminated raw milk can become a source of pathogenic bacteria to humans. In 13% of the samples, TPC in cow's milk ( $1.0 \times 10^3$  to  $3.0 \times 10^7$  CFU/ml) exceeded the geometric means of microorganism content laid down by legislation, during a period of two months, with at least two samples per month (Regulation (EC) No. 853/2004).

Table 1 and Figure 2 show that the average TPC value is significantly associated with the origin of milk ( $P < 0.01$ ; Kruskal-Wallis test). Whilst the lowest values have again been found in cow's milk ( $1.0 \times 10^3$ – $3.0 \times 10^7$  CFU/ml), the highest TPC values were found in goat's milk ( $9.3 \times 10^8$ – $1.2 \times 10^9$  CFU/ml), similarly like SCC values. Subsequent tests showed

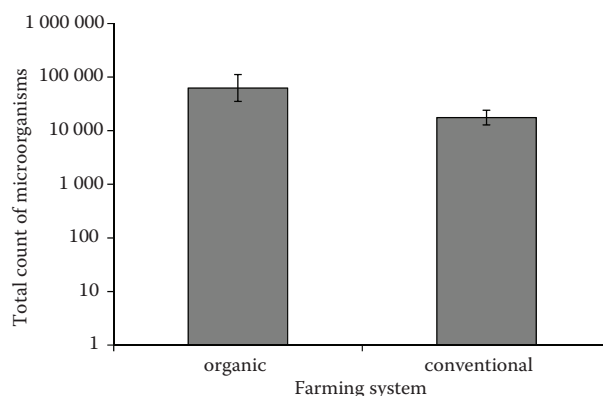


Figure 3. Total plate count (TPC) of microorganisms in cow's raw milk according to the type of farming

The columns represent geometric means of TPC, vertical bars correspond to 95% confidence intervals of the geometric means

that statistically significant differences exist mainly between cow's milk on the one hand and sheep's and goat's milk on the other hand (see Dunn's post-hoc tests). However, no statistically significant difference in TPC values between goat's and sheep's milk was detected. In a study of MUEHLHERR *et al.* (2003) the authors reported in small ruminants the TPC values of 4.70 log CFU/ml (min. 2.00 log CFU/ml and max. 8.64 log CFU/ml). For goat's milk, the average TPC value was 4.69 log CFU/ml and in sheep's milk it was slightly higher, namely 4.78 log CFU/ml.

The microbiological quality of raw milk can be affected by several factors, such as milking, housing, farming system (organic, conventional), and the season of the year. Table 2 and Figure 3 show that while the season of the year does not statistically significantly affect the average values of TPC in bovine milk ( $P > 0.05$ ; Kruskal-Wallis test), the difference between conventional and organic farms is statistically significant ( $P < 0.01$ ; Mann-Whitney test) – in conventional systems the average TPC value is lower than that of organic farming.

TPC values in the summer months ranged from  $1.8 \times 10^3$  to  $1.8 \times 10^6$  CFU/ml, but in September and October, the TPC ranged from  $2.0 \times 10^3$  to  $3.1 \times 10^7$  CFU/ml. These results that correlate with the results of other authors point to the fact that the limit value of TPC is sometimes exceeded in milk samples. Between 1993 and 1996, a study carried out in the USA investigated the microbiological quality of bulk milk samples: TPC in these samples ranged from  $1.0 \times 10^5$  CFU/ml to more than  $5.0 \times 10^6$  CFU/ml



doi: 10.17221/25/2016-CJFS

Table 2. Descriptive statistics for total plate count (TPC) in cow's raw milk according to farming type and season

Statistics	Farming system		Season of the year			
	organic	conventional	spring	summer	autumn	winter
<i>n</i>	44	110	49	44	45	16
Minimum	$3.0 \times 10^3$	$1.0 \times 10^3$	$1.0 \times 10^3$	$1.8 \times 10^3$	$2.0 \times 10^3$	$2.3 \times 10^3$
Maximum	$3.0 \times 10^7$	$2.2 \times 10^6$	$2.0 \times 10^6$	$1.8 \times 10^6$	$3.1 \times 10^7$	$2.7 \times 10^5$
Geometric mean	$6.2 \times 10^4$	$1.8 \times 10^4$	$2.4 \times 10^4$	$1.9 \times 10^4$	$4.2 \times 10^4$	$1.5 \times 10^4$
95% confidence interval	$3.5 \times 10^4$	$1.3 \times 10^4$	$1.4 \times 10^4$	$1.2 \times 10^4$	$2.2 \times 10^4$	$8.1 \times 10^3$
Of geometric mean	$1.1 \times 10^5$	$2.4 \times 10^4$	$4.2 \times 10^4$	$3.0 \times 10^4$	$7.8 \times 10^4$	$2.9 \times 10^4$
Median	$4.5 \times 10^4$	$1.3 \times 10^4$	$1.8 \times 10^4$	$1.6 \times 10^4$	$2.9 \times 10^4$	$1.4 \times 10^4$
Lower quartile	$1.8 \times 10^4$	$5.3 \times 10^3$	$7.2 \times 10^3$	$4.9 \times 10^3$	$7.2 \times 10^3$	$6.9 \times 10^3$
Upper quartile	$2.0 \times 10^5$	$4.7 \times 10^4$	$9.7 \times 10^4$	$5.0 \times 10^4$	$1.4 \times 10^5$	$2.4 \times 10^4$
Interquartile range	$1.3 \times 10^5$	$4.1 \times 10^4$	$9.0 \times 10^4$	$4.5 \times 10^4$	$1.3 \times 10^5$	$1.7 \times 10^4$
Statistical significance	$P < 0.01^a$		$P > 0.05^b$			

<sup>a</sup>Mann-Whitney test; <sup>b</sup>Kruskal-Wallis test; *n* – No. of samples

(BOOR *et al.* 1998). Significantly higher counts were reported in cattle in developing countries, e.g. in Sudan between 2003 and 2004 ( $4.0 \times 10^5$ – $3.3 \times 10^{11}$  CFU/ml) (IBTISAM *et al.* 2007).

This study included the detection of bacteria from the family *Enterobacteriaceae*. The values of *Enterobacteriaceae* counts ranged between  $1.0 \times 10^1$  and  $2.0 \times 10^6$  CFU/ml. The study of IBTISAM *et al.* (2007) reported *Enterobacteriaceae* counts in the range from 0 to  $1.5 \times 10^{10}$  CFU/ml. These authors analysed 120 milk samples from 60 farms in Sudan in 2003–2004. The presence of these bacteria, as well as the presence of enterococci (ranged from  $1.0 \times 10^1$  to  $1.6 \times 10^5$  CFU/ml) indicates the potential faecal contamination during milking (IBTISAM *et al.* 2007). At present, there are no legislative limits for the family *Enterobacteriaceae* and enterococci, both groups of bacteria are indicators of hygienic conditions in primary milk production.

*E. coli* bacteria are considered an important hygiene indicator throughout the process of raw milk obtaining, storage, transport, and sale. *E. coli* is commonly found in the intestinal microflora of humans and warm-blooded animals, but it may become a pathogenic organism (COSTA *et al.* 2009). In heat-untreated raw materials of animal origin, such as raw milk and meat, *E. coli* occurs quite frequently (BADRI *et al.* 2009). No limit value of this indicator for raw milk has been laid down by any European regulation, but the up to now valid standard ČSN 57 0529:1993 sets the limit value for cow's milk at  $1.0 \times 10^3$  CFU/ml. Values detected in particular months ranged from  $1.0 \times 10^1$  to

$4.0 \times 10^6$  CFU/ml. The lowest values of *E. coli* were found in cow's milk ( $1.0 \times 10^1$  to  $2.0 \times 10^3$  CFU/ml), whilst for small ruminant milk, the values of *E. coli* were twice as high (sheep milk  $1.0 \times 10^1$  to  $4.0 \times 10^6$  CFU/ml, goat milk  $1.0 \times 10^1$  to  $1.6 \times 10^6$  CFU/ml). The Regulation limit was exceeded in milk collected on two farms (4.9%), one producing cow's milk and the other sheep's and goat's milk. However, the limit which may be due to e.g. animal housing system and hygienic level of animal husbandry practices was increased only sporadically. Raw milk can get contaminated via the intramammary route during clinical or subclinical mastitis or, which is more common, raw milk can be contaminated directly with animal faeces or indirectly by the staff or from the environment, including milking equipment and other contaminated tools used in various phases of milk obtaining (ALTALHI & HASSAN 2009). *E. coli* counts exceeded the limit of  $1.0 \times 10^3$  CFU/ml only in three samples (1.2%) of cow's raw milk. In the study of PYZ-ŁUKASIK *et al.* (2015), *E. coli* counts were significantly lower, ranging from  $5.0 \times 10^0$  to  $1.1 \times 10^2$  CFU/ml. Coliform bacteria, including *E. coli*, are natural components of milk, and seldom are referred to as the causative agent of mastitis. A comparison of the detected *E. coli* counts with other publications is problematic, because *E. coli* counts are not commonly determined, except for coliform bacteria, which are normally in the range from 0 to  $1.5 \times 10^{10}$  CFU/ml. Raw milk and its products can become a source of not only commensal *E. coli*, but also of pathogenic serotypes, including *E. coli* O157: H7 (BADRI *et al.* 2009; GIACOMETTI *et al.* 2013). In our study, vero-

Table 3. The occurrence of *Staphylococcus aureus* depending on the season in cow's raw milk

Season	No. of examined samples	<i>S. aureus</i>
Spring	56	13 (23.2%)
Summer	47	13 (27.7%)
Autumn	53	13 (24.5%)
Winter	19	8 (42.1%)
Total	175	47 (26.9%)

toxigenic *E. coli* were detected in 3 samples (1.3%) (cow's milk 0%, goat's milk 6.3%, sheep's milk 4.4%).

*Staphylococcus aureus* is responsible for both clinical and subclinical mastitis (BERGONIER *et al.* 2003). Such infections result in significant economic losses due to reduced milk production, and constitute potential sources of foodborne intoxication for consumers. The occurrence of *S. aureus* in raw milk ranges from 16.7% to 96.2% (MUEHLHERR *et al.* 2003; JØRGENSEN *et al.* 2005; CHU *et al.* 2012; SPANU *et al.* 2013). In our study, the presence of *S. aureus* was confirmed in 29.1% of samples (cow's 26.9%; goat's 34.4%; sheep's milk 39.1%), but the counts were either negative or less than  $5.0 \times 10^2$  CFU/ml. *S. aureus* counts in raw goat and sheep milk are consistent with the results of MUEHLHERR *et al.* (2003) in Switzerland where *Staphylococcus aureus* was found in 109 samples of goat's milk (31.7%) and in 21 samples of sheep's milk (33.3%). *S. aureus* counts found in cow's raw milk in our study were slightly lower (26.9%). However, the results are consistent with the study of JAMALI *et al.* (2015) in which *S. aureus* was detected in 328 samples (12.4%). In this study, *S. aureus* exceeded the limit values specified in Decree No. 289/2007 Coll., in four samples (1.6%). Currently, there are no cow's milk limits set down in the European Community regulations. The national limit for the occurrence of *Staphylococcus aureus* in raw milk is  $5.0 \times 10^2$  CFU/ml. The highest numbers of *S. aureus* in samples of cow's raw milk were recorded in the winter, but no statistically significant association between the season and the presence of *S. aureus* was demonstrated ( $P > 0.05$ ; Chi-squared test for independence). More detailed information is provided in Table 3.

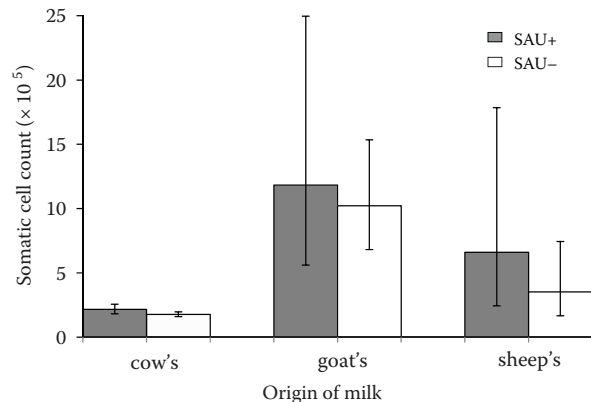


Figure 4. The number of somatic cells according to the finding of *Staphylococcus aureus* (SAU) and origin of milk. The columns represent values of geometric means of PSB, vertical line correspond to 95% confidence intervals of the geometric means

Increasing *S. aureus* counts were observed especially in samples with higher SCC counts. As shown in Figure 4, it is evident that PSB is higher in samples with proven *S. aureus* (SAU), both cow's and goat's milk or sheep's milk. The differences are not statistically significant ( $P > 0.05$ ; Mann-Whitney U test). The occurrence of *S. aureus* was statistically significantly associated with the animal housing system ( $P < 0.01$ ; Fisher's exact test). It is almost 3 times higher for organic farming than for conventional farming. The OR (odds ratio) value is 4.861, which means that the chance of finding *S. aureus* in herds in organic farming is almost 5 times higher than in conventional herds. VYLETĚLOVÁ and GENČUROVÁ (2007) documented the increased occurrence of *S. aureus* in herds on organic farms (38.5%) in comparison with conventional herds (28.1%).

It follows from Figure 4 that for obtaining high probabilities of positive findings of *S. aureus*, SCC values would have to exceed  $10^6$ /ml. However, it should be taken into consideration that the model was based on a low number of samples with positive finding of *S. aureus* (25.5%).

The occurrence of *Listeria monocytogenes* poses a potential risk to consumers. The bacteria have al-

Table 4. Findings of *Staphylococcus aureus*, verotoxigenic *Escherichia coli* (VTEC), and *Listeria monocytogenes* (LM)

Milk	No. of samples	<i>S. aureus</i>	VTEC	LM
Cow's	175	47 (26.9%)	0 (0%)	1 (0.6%)
Goat's	32	11 (34.4%)	2 (6.3%)	1 (3.1%)
Sheep's	23	9 (39.1%)	1 (4.4%)	1 (4.4%)
Total	230	67 (29.1%)	3 (1.3%)	3 (1.3%)

doi: 10.17221/25/2016-CJFS

ready been isolated from raw milk and dairy products throughout the world (LYYTIKAINEN *et al.* 2000; DONNELLY 2001; LUNDÉN *et al.* 2004). In our study, *L. monocytogenes* was detected in 3 samples of raw milk (1.3%) (0.6% cow's milk, 3.1% goat's milk, and 4.4% sheep's milk). Our results correlate with the results of other authors who reported the occurrence of *Listeria monocytogenes* up to 7.1% (JAKOBSEN *et al.* 2011; HILL *et al.* 2012; RUUSUNEN *et al.* 2013).

The occurrence of the other pathogenic bacteria of the genera *Salmonella* spp. and *Campylobacter* spp. was not confirmed in the samples of raw milk collected in this study. The occurrence of these pathogens is dependent on the health status of animals and varies significantly between individual farms (JAYARAO *et al.* 2006). Some authors reported the presence of these microorganisms in milk. VAN KESSEL *et al.* (2004) detected 9 *Salmonella* serotypes in 2.6% ( $n = 821$ ) samples of raw milk collected in 21 USA states, KARPÍŠKOVÁ *et al.* (2011) 3 serotypes in 3.2% ( $n = 219$ ) samples of cow's raw milk.

## CONCLUSION

Between 2012 and 2014, monitoring the quality of cow's raw milk in the Czech Republic was mainly focused on SCC, and the quantitative and qualitative microbiological parameters in raw milk. The limit of parameters for raw milk set down by authorities was exceeded in 13% of samples. Pathogenic microorganisms such as verotoxigenic *Escherichia coli*, *Staphylococcus aureus*, and *Listeria monocytogenes* were also detected in the collected milk samples.

Therefore, the study confirmed that unpasteurised raw milk may pose a health risk to consumers, particularly if the producers do not abide by the recommendations of handling, storage and expiration date. Currently, pasteurisation is the best solution, ensuring safety of this commodity.

## References

- Altalhi A.D., Hassan S.A. (2009): Bacterial quality of raw milk investigated by *Escherichia coli* and isolates analysis for specific virulence-gene markers. *Food Control*, 20: 913–917.
- Badri S., Filliol I., Carle I., Hassar M., Fassouane A., Nozha C. (2009): Prevalence of virulence genes in *Escherichia coli* isolated from food in Casablanca (Morocco). *Food Control*, 20: 560–564.
- Bergonier D., De Cremoux R., Rupp R., Lagriffoul G., Berthelot X. (2003): Mastitis of dairy small ruminants. *Veterinary Research*, 34: 689–716.
- Boor K.J., Brown D.P., Murphy S.C., Kozlowski S.M., Bandler D.K. (1998): Microbiological and chemical quality of raw milk in New York State. *Journal of Dairy Science*, 81: 1743–1748.
- Bufano G., Dario C., Laudadio V. (1996): The characterisation of Leccese sheep: variations of chemical composition and lactodynamographic parameters in milk as related to somatic cell counts. In: *Somatic Cells and Milk of Small Ruminants*. Book Series: EAAP Publication No. 77. Wageningen, Wageningen Pers: 301–304.
- Costa D., Vinué L., Poeta P., Coelho A.C., Matos M., Sáenz Y., Somalo S., Zarazaga M., Rodrigues J., Torres C. (2009): Prevalence of extended-spectrum beta-lactamase-producing *Escherichia coli* isolates in faecal samples of broilers. *Veterinary Microbiology*, 138: 339–344.
- Chu C., Yu C., Lee Y., Su Y. (2012): Genetically divergent methicillin-resistant *Staphylococcus aureus* and *sec* dependent mastitis of dairy goats in Taiwan. *BMC Veterinary Research*, 8: 39.
- Decree No. 289/2007 Coll. (2007): On veterinary and sanitary requirements for animal products that are not regulated by directly applicable regulations of the European Communities. Collection of Laws 289/2007.
- Donnelly C. (2001): Factors associated with hygienic control and quality of cheeses prepared from raw milk: a review. *Bulletin of the International Dairy Federation*, 369: 16–27.
- EFSA-ECDC (2012): Scientific report of EFSA and ECDC: The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2010. *EFSA Journal*, 10: 2597.
- Fagan P.K., Hornitzky M.A., Bettelheim K.A., Djordjevic S.P. (1999): Detection of shiga-like toxin (*stx1* and *stx2*), intimin (*eaeA*), and enterohemorrhagic *Escherichia coli* (EHEC) hemolysin (EHEC *hlyA*) genes in animal feces by multiplex PCR. *Applied and Environmental Microbiology*, 65: 868–872.
- Fthenakis G.C., el-Masannat A.T.S., Booth J.M., Jones J.E.T. (1991): Somatic cell count of ewe's milk. *British Veterinary Journal*, 147: 575–581.
- Giacometti F., Serraino A., Finazzi G., Daminelli P., Losio M.N., Arrigoni N., Piva S., Florio D., Riu R., Zanoni R.G. (2013): Sale of raw milk in northern Italy: food safety implications and comparison of different analytical methodologies for detection of foodborne pathogens. *Foodborne Pathogens and Disease*, 9: 293–297.
- González-Rodríguez M.C., Gonzalo C., San Primitivo F., Carmenes P. (1995): Relationship between somatic cell

- count and intramammary infection of the half udder in dairy ewes. *Journal of Dairy Science*, 78: 2753–2759.
- Hill B., Smythe B., Lindsay D., Sheperd J. (2012): Microbiology of raw milk in New Zealand. *International Journal of Food Microbiology*, 157: 305–308.
- Ibtisam E., El Zubeir M., Ahmed Mahbora I.A. (2007): The hygienic quality of raw milk produced by some dairy farms in Khartoum State, Sudan. *Research Journal of Microbiology*, 2: 988–991.
- Jakobsen R.A., Heggebø R., Sunde E.B., Skjervheim M. (2011): *Staphylococcus aureus* and *Listeria monocytogenes* in Norwegian raw milk cheese production. *Food Microbiology*, 28: 492–496.
- Jamali H., Paydar M., Radmehr B., Ismail S., Dadrasnia A. (2015): Prevalence and antimicrobial resistance of *Staphylococcus aureus* isolated from raw milk and dairy products. *Food Control*, 54: 383–388.
- Jayarao B.M., Donaldson S.C., Straley B.A., Sawant A.A., Hegde N.V., Brown J.L. (2006): A survey of foodborne pathogens in bulk tank milk and raw milk consumption among farm families in Pennsylvania. *Journal of Dairy Sciences*, 89: 2451–2458.
- Jørgensen H.J., Mørk T., Høgasen H.R., Rørvik L.M. (2005): Enterotoxigenic *Staphylococcus aureus* in bulk milk in Norway. *Journal of Applied Microbiology*, 99: 158–166.
- Karpíšková R., Kolářčková I., Vyleťelová M., Janštová B. (2011): Studie „Mléčné automaty“ – nálezy původců alimentárních onemocnění v syrovém mléce. *Zprávy centra epidemiologie a mikrobiologie*, 20/ 6: 212–214.
- Lundén J., Tolvanen R., Korkeala H. (2004): Human listeriosis outbreak slinked to dairy products in Europe. *Journal of Dairy Science*, 87: 6–11.
- Lyytikäinen O., Autio T., Maijala R., Ruutu P., Honkanen-Buzalski T., Miettinen M., Hatakka M., Mikkola J., Anttila V.J., Johansson T., Rantala L., Aalto T., Korkeala H., Siitonen A. (2000): An outbreak of *Listeria monocytogenes* serotype 3a infections from butter in Finland. *The Journal of Infectious Diseases*, 181: 1838–1841.
- Martineau F., Picard F.J., Roy P.H., Ouellette M., Bergeron M.G. (1998): Species-specific and ubiquitous DNA-based assays for rapid identification of *Staphylococcus aureus*. *Journal of Clinical Microbiology*, 36: 618–623.
- Muehlherr J.E., Zweifel C., Corti S., Blanco J.E., Stephan R. (2003): Microbiological quality of raw goat's and ewe's bulk-tank milk in Switzerland. *Journal of Dairy Science*, 86: 3849–3856.
- Oliver S.P., Boor K.J., Murphy S.C., Murinda S.E. (2009): Food safety hazards associated with consumption of raw milk. *Foodborne Pathogens and Disease*, 6: 793–806.
- Pyz-Łukasik R., Paszkiewicz W., Tatara M.R., Brodzki P., Bełkot Z. (2015): Microbiological quality of milk sold directly from producers to consumers. *Journal of Dairy Science*, 15: 299–234.
- Regulation (EC) No 853/2004 of The European Parliament and of The Council of 29 April 2004 laying down specific hygiene rules for on the hygiene of foodstuffs. *Official Journal of the European Union*, L 139/55.
- Ruusunen M., Salonen M., Pulkkinen H., Huuskonen M., Hellström S., Revez J., Hänninen M.L., Fredriksson-Ahomaa M., Lindström M. (2013): Pathogenic bacteria in Finnish bulk tank milk. *Foodborne Pathogens and Disease*, 10: 99–106.
- Spanu V., Scarano C., Virdis S., Melito S., Spanu C., De Santis E.P. (2013): Population structure of *Staphylococcus aureus* isolated from bulk tank goat's milk. *Foodborne Pathogens and Disease*, 10: 310–315.
- Van Kessel J.S., Karns J.S., Gorski L., McCluckes B.J., Perdue M.L. (2004): Prevalence of *Salmonellae*, *Listeria monocytogenes* and fecal coliforms in bulk tank milk on U.S. dairies. *Journal of Dairy Science*, 87: 2822–2830.
- Verraes C., Vlaemynck G., Van Weyenberg S., De Zutter L., Daube G., Sindic M., Uyttendaele M., Herman L. (2015): A review of the microbiological hazards of dairy products made from raw milk. *International Dairy Journal*, 50: 32–44.
- Vyleťelová M., Genčurová V. (2007): Comparison of food pathogens occurrence in raw milk between ecological and conventional farms. In: *Proceedings Congress HELEXPO*, Mar 9–11, 2007, Thessaloniki, Greece. III: 339–343.

Received: 2016–01–26

Accepted after corrections: 2016–05–23

Published online: 2016–06–01

---

*Corresponding author:*

Mgr. KATEŘINA BOGDANOVIČOVÁ, Veterinární a farmaceutická univerzita Brno, Fakulta veterinární hygieny a ekologie, Palackého tř. 1946/1, 612 42 Brno, Česká republika; E-mail: bogdanovicovak@vfu.cz

---