JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

The prevalence of *Listeria monocytogenes* and *Staphylococcus aureus* and their virulence genes in bulk tank milk in Kosovo

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Abstract
Introduction: Milk is considered to be a healthy, nutritious food product. Microbiological quality is an important aspect in evaluating the quality of milk.
Methodology: A total of 603 bulk tank milk samples from 221 farms distributed across ten different regions were collected for milk quality assessment. Quality was judged by total viable count, and the prevalence of two foodborne pathogens (*Listeria monocytogenes* and *Staphylococcus aureus*) by using selective media and 16S rRNA gene sequencing. The presence of virulence genes was detected by polymerase chain reaction (PCR) using specific primers.
Results: Milk from only 7% (15/221) of farms were found to comply with the European Union standard. Interestingly, the microbiological quality of milk from the larger herd size farms (more than 10 cows) was better than in smaller herds. *L. monocytogenes* was found in 2.7% (6/221) of farms, and all the examined *L. monocytogenes* isolates were positive with respect to the virulence genes *prfA*, *actA*, and *hlyA*. *S. aureus* was found in 39.8% (88/221) of the farms. In total, 30.7% (27/88) of the staphylococci were positive for enterotoxin production. The enterotoxins identified were toxin B (40.7%), toxin D (33.4%), toxin C (18.5%), and toxin A (7.4%).
Conclusions: The total number of bacteria in milk was very high. The presence of two foodborne pathogens in raw milk represents a great health risk to consumers. To improve the microbial quality of milk in Kosovo, important measures to improve hygiene, including better information, guidance, and control, are needed.

Key words: milk quality; *Listeria*; *Staphylococcus*; enterotoxin; virulence.

J Infect Dev Ctries 2017; 11(3):247-254. doi:10.3855/jidc.8256

(Received 15 February 2016 – Accepted 05 July 2016)

Introduction

Milk is a nutritious food product for humans, and it is obtained from a variety of animal sources, such as cows, goats, sheep, and buffaloes. Bovine milk contains lipids, proteins (caseins, whey), carbohydrates (lactose), amino acids, vitamins, and minerals (particularly calcium) essentials of good nutrition [1]. However, due to its nutritious properties, milk is also known as an excellent growth environment for both non-pathogenic and pathogenic bacteria [1,2]. *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli*, which are normally considered significant pathogens, are often found in milk or milk products in both industrialized and developing countries [3-5]. *L. monocytogenes* can cause meningitis, septicemia, and abortion in humans and animals [6-8]. *S. aureus* is the main cause of subclinical mastitis in animals, and some strains have the ability to produce toxins, especially when present in high cell numbers (> 10^5 colony-forming units (cfu)/mL). Staphylococcal food infection in humans is often accompanied by vomiting, diarrhea, and abdominal cramps [9,10]. The causes of contamination are many, including loss of udder integrity of infected animals, poor hygiene, or practices leading to further spreading the pathogens in farms, milking production facilities, and during the milking process [11]. Numerous studies have shown that factors such as animal health, lactation, season, climate, animal breed, feeding practice, housing, hygiene of premises, milking practice and storage, and milking facilities and equipment play important roles in the quality of bulk tank milk [12-16].

Kosovo is a young and small country located in the Balkan region. Milk, which is an important part of daily nutrition in the country, is produced in a quantity of approximately 257,500 tons per year [17]. The majority of dairy farms (94%) in Kosovo are traditional type (also called subsistence farms), 5% of the farms are semi-commercial farms, and only 1% are commercial farms; the differentiation between the latter two is based on the number of cows present on the farm [17,18]. Subsistence farmers produce milk mostly for their own consumption, while semi-commercial or commercial farmers sell their milk at small nearby dairy factories or at local markets. In European countries, the milk quality is much better compared to that in developing countries. This may be due to more rigorous inspection control practices from authorities, better managing of the farms, better hygiene, and better infrastructure in general [19,20]. To our knowledge, no systematic microbial analysis has been done on bulk tank milk in Kosovo, especially with regard to the problem of foodborne pathogens. Hygiene and good management of bulk tank milk and its products are some of the important aspects in achieving high microbiological quality, and this issue needs to be addressed before Kosovo can export dairy products, especially to the European Union market, where adherence to high quality standards is required. In this study, we systematically evaluated the microbiological quality of raw cow milk in Kosovo in general and with special focus on the prevalence of two foodborne pathogens (*L. monocytogenes* and *S. aureus*).

Methodology

Sample collection

Between December 2011 and June 2012, bulk tank cow milk samples were collected from 221 farms in Kosovo. These farms were chosen from ten different regions: Decan (15 farms), Ferizaj (26 farms), Gjakova (15 farms), Istog (21 farms), Lipjan (20 farms), Podujeva (15 farms), Rahovec (60 farms), Skenderaj (20 farms), Sharr (16 farms), and Theranda (13 farms). To obtain a reliable and consistent dataset, milk samples were collected every second month and three times from each farm except for the farms from Rahovec, where samples were collected only at two time points due to technical problems. This yielded 603 samples in total. The samples were kept in sterile plastic tubes and transported in an ice box (4°C–8°C) to the laboratory within 4 hours of pick-up at collection points, and the samples were immediately processed for further analyses. To collect information about herd size, milking system, cow breed, and health status, a questionnaire was prepared and completed during personal interviews with the owners.

Standard plate counting (SPC)

Total viable counts (TVCs) of microorganisms were determined following the guidelines of International Standards of Organization (ISO 4833:2003) using plate count agar (PCA) [21]. Briefly, collected samples were serially diluted and 100 μL from higher dilutions (10^{-8} to 10^{-2}) was plated on PCA plates (Oxoid, Basingstoke, United Kingdom) and incubated aerobically for 72 hours at 30°C before total colony-forming units were determined.

Isolation and enumeration of S. aureus

Isolation of *S. aureus* from bulk tank milk was performed according to the international standard procedure (ISO 6888-1:1999) using Baird-Parker agar supplemented with egg yolk tellurite emulsion (Oxoid, Basingstoke, United Kingdom) [22]. The plates were incubated under aerobic conditions at 37°C for 24 to 48 hours. From each milk sample, five randomly selected egg yolk reaction-positive colonies (an indication of *S. aureus*) and five egg yolk reaction-negative isolates (for other *Staphylococcus* spp.) were selected for further characterization. All isolates were tested for Gram staining and catalase activity, hemolytic properties, and their ability to coagulate rabbit plasma (tube coagulase test) and to produce clumping factor (Staphylase test; Oxoid, Basingstoke, United Kingdom). The strains that showed similarity were grouped, and only one isolate from each group was frozen in stab agar and further analyzed. Colonies were re-grown on brain-heart infusion (BHI) broth and stored at -80°C with 13% glycerol.

DNA isolation, PCR, and DNA sequencing

Bacterial genomic DNA was extracted by using a modified purification protocol described previously [23]. PCR was done on genomic DNA to amplify 16S rRNA genes, using appropriate primers described previously (Table 1) [24,25]. The thermal profile used for PCR was at 90°C for 1 minute, and 35 cycles at 95°C for 15 seconds, 54°C for 30 seconds, and 70°C for 90 seconds. The PCR product was purified by using a QIA quick PCR purification kit (Qiagen, Hilden, Germany), and sequenced by an ABI Prism 377 DNA sequencing system (Applied Biosystems, Darmstadt, Germany).

Determination of staphylococcal toxin production

Detection of staphylococcal enterotoxins A, B, C, and D was performed using the reverse passive latex agglutination kit for enterotoxin (SET-RPLA) (Oxoid, Basingstoke, United Kingdom). Before testing, each colony was plated in Baird-Parker agar and incubated for 24 hours at 37°C under aerobic conditions. One colony per each sample where *S. aureus* was found at more than 10^3 cfu/mL (88 isolates) was used in toxin detection. The pure cultures were regrown on tryptic soya broth (Oxoid, Basingstoke, United Kingdom) following manual instructions of the SET-RPLA kit without quantification (Oxoid, Basingstoke, United Kingdom).

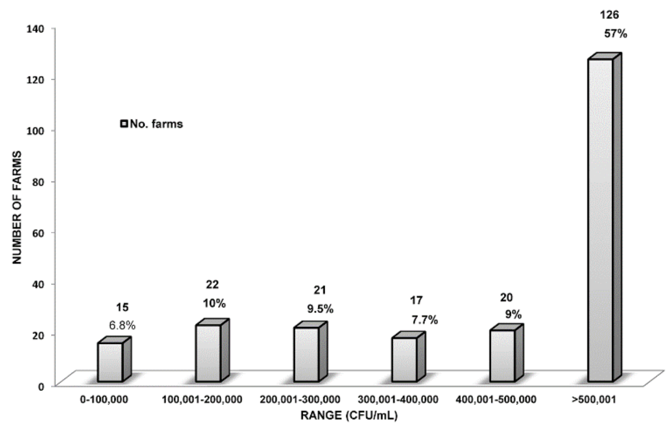
Isolation and enumeration of L. monocytogenes

Isolation of *L. monocytogenes* from raw milk was performed according to the EN ISO 11290-2 standard procedure of the International Organization for Standardization [26]. Raw milk samples were plated in triplicate directly onto Oxford agar and PALCAM agar plates (Sigma-Aldrich, St. Louis, United States). Plates were incubated at 37°C and scored for presumptive *Listeria* spp. Colony hydrolysis, black colonies (indication for *Listeria* spp.) were observed at 24 and 48 hours. Presumptive *Listeria* isolates were then grown in BHI broth (Oxoid, Basingstoke, United Kingdom) for further testing, such as oxidase with 1% tetramethyl-p-phenylenediamine dihydrocholride, catalase with 3% hydrogen peroxide, and Gram staining using a three-step staining kit (Oxoid, Basingstoke, United Kingdom). Hemolytic activity was determined by stabbing blood agar (Columbia with 5% sheep blood; Thermo Fisher Scientific, Lenexa, Kansas, United States), which was incubated at 37°C for 48 hours. Single colonies were regrown in BHI broth (Oxoid, Basingstoke, United Kingdom) and stored at -80°C with 13% glycerol.

Virulence gene detection

The following virulence genes were detected: *prfA* encoding a positive regulatory factor A; *hyl* encoding a pore-forming toxin (listeriolysin O, LLO); and *actA*, encoding an actin assembly-inducing protein. Primers are listed in Table 1. Confirmatory identification of *L. monocytogenes* was performed by 16S rRNA gene sequencing.

Figure 1. Total viable counts in raw milk from 221 farms in Kosovo.



Statistical analysis

Statistical analysis of TVC and the prevalence of the two foodborne pathogens (*S. aureus* and *L. monocytogenes*) were carried out with R software, version 3.1.0. To evaluate the significant differences and mean values, two-way analysis of variance (ANOVA) was applied, followed by Tukey's test. Statistical significance was defined when a p value was below 0.005.

Results

Enumeration of microorganisms

In total, 603 raw milk samples were collected during the period of December 2011 to June 2012. The samples were derived from 221 farms distributed in 10 different regions. In most cases, the samples were collected 3 times from each farm with 2–3 months between the time points. For the farms from the region of Rahovec, the samples were collected only twice due to technical problems with transport. The first sampling was carried out during December and January, the second sampling during February and March, and the third one during May and June. Most of the farms

Table 1. DNA primers used in PCR for detection of virulence genes in *Listeria monocytogenes* and 16s rRNA gene sequencing.

Name	Sequence (5-3)	Target gene	Reference
11F	F: TAACACATGCAAGTCGAACG	16S rRNA	[24]
5R	R: GGTTACCTTGTTACGACTT		[25]
prfA	F: AACCAATGGGATCCACAAG	*prfA*	[27]
prfA	R: ATTCTGCTAACAGCTGAGC		
actA	F: TAGCGTATCACGAGGAGG	*actA*	[27]
actA	R: TTTTGAATTTCATATCATTCACC		
hylA	F: CAAACTGAAGCAAAGGATGCA	*hylA*	[27]
hylA	R: CTAATGTATTTACTGCGTTGTTA		

F: forward primer; R: reverse primer

included in this study were commercial farms (185/221) that had more than 5 cows per farm (Supplementary Table 1). The TVCs varied considerably between the farms, ranging from 10^4 to 10^7 cfu/mL of milk. Only 6.8% of the farms (15 of 221) complied with the accepted values of the EU (< 105 cfu/mL), but 43% of the farms fulfilled the less strict regulation of Ministry of Agriculture, Forestry and Rural Development of Kosovo (MAFRED) (< 5 × 10^5 cfu/mL milk) (Figure 1). Within the individual farms, only small differences (10^2 to 10^3 cfu/mL) in TVCs were found between samples collected at different time points. The high differences in TVCs were observed between different farms (Supplementary materials Table 1).

To analyze the effect of herd size on TVCs, the farms were divided into three groups based on the number of cows per farm: small (1–5 cows), medium (5–10 cows), and large size (> 10 cows). Significant differences between these three groups were observed (Figure 2). In large farms (< 10 cows), the TVC values were lower than in medium and small farms, approximately 1 × 10^5 cfu/mL, 9 × 10^5 cfu/mL, and 1.4 × 10^6 cfu/mL, respectively (Figure 2). Similarly, differences between regions were also observed. The highest TVC numbers were found in the following three regions: Rahovec (25 × 10^5cfu/mL), Gjakova (2.1 × 10^6 cfu/mL), and Ferizaj (1 × 10^6 cfu/mL) (Figure 3).

Staphylococcus spp. in raw bulk milk

S. aureus is frequently found in raw milk in most European countries, occurring at a level of between 18% to 75% in different countries [28-30]. In the samples from Kosovo, the presence of *S. aureus* varied between 10^2 and 10^6 cfu/mL. TVCs below 2 × 10^3 cfu/mL were found in 60.2% of the farms (133/221), while TVCs above 2 × 10^3cfu/mL were found in 39.8% of the farms (88/221) (Table 2).

Based on the results obtained from the coagulase test, milk from 88 farms was apparently contaminated with *S. aureus*, while milk from 44 farms was

Figure 2. Mean value of total viable counts in raw milk from different group of farms in three different periods.

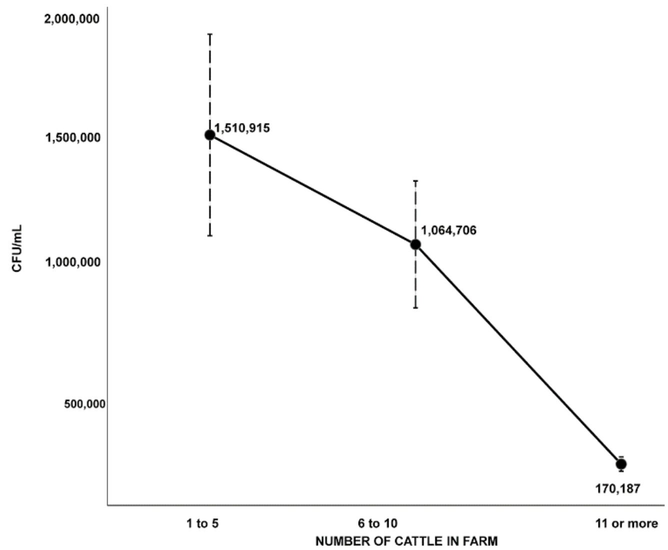
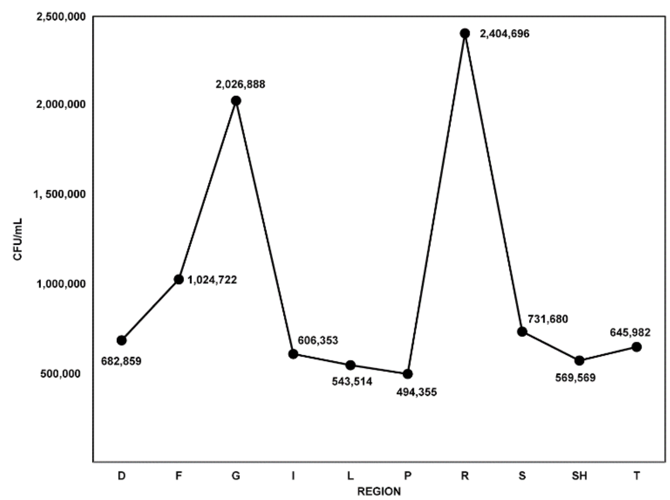


Figure 3. Mean value of total viable counts in raw milk from different regions.



D: Decani; R: Rahovec; S: Skenderaj; F: Ferizaj; P: Podujeva; SH: Sharr; I: Istog; TH: Theranda; L: Lipjan; G: Gjakova

Table 2. Presence of *Staphylococcus* spp. and *Listeria* spp. in raw bulk milk.

Species	Farms	Range (cfu/mL)		
		10^0–10^2	10^3–10^5	> 10^5
Staphylococcus aureus	88	N	52	36
Staphylococcus infantarius	23	N	19	4
Staphylococcus simulans	15	N	13	2
Staphylococcus epidermidis	4	N	4	0
Listeria monocytogenes	6	4	2	0
Listeria innocua	4	1	3	0
Listeria seelingeri	2	1	1	0
Listeria grayi	1	0	1	0

N: not analyzed

Table 3. Virulence genes in *L. monocytogenes* identified by PCR.

Bacterial isolates/strains	PCR result		
	prfA	*actA*	*inlA*
Listeria monocytogenes:Kosovo strains (KS7001–KS7006)	+	+	+
Listeria monocytogenes LMGT 2651[a]	+	+	+
Listeria innocua LMGT2710 [a]	-	-	-
Enterococcus faecalis V583[b]	-	-	-

[a] positive control samples; [b] negative control samples

contaminated with other *Staphylococcus* spp., either *S. simulans*, *S. epidermidis*, or *S. infantarius* (Table 2).

From farms associated with high levels of staphylococci, one random staphylococcal isolate was assessed for enterotoxin (*i.e.*, 88 staphylococcal isolates from 88 different farms). It was found that 40 of 88 isolates (36 *S. aureus* and 4 other *Staphylococcus* spp.) were obtained from samples with staphylococci higher than 10^5 cfu/mL, thus representing a potential risk of toxin production. These isolates were mainly found in the farms in Rahovec (n = 12), Skenderaj (n = 6), Ferizaj (n = 5), Podujeva (n = 4), Sharr (n = 3), Istog (n = 3), Theranda (n = 2), Lipjan (n = 2), Gjakova (n = 2), and Decani (n = 1) (Supplementary Table 1). *Staphylococcus* species are known to produce different heat-stable enterotoxins (A, B, C, D, and E) [28]. Using the SET-RPLA kit, 27 of 88 (30.7%) isolates were found to produce enterotoxins. The enterotoxins identified were toxin B in 40.7% (11/27), toxin D in 33.4% (9/27), toxin C in 18.5% (5/27), and toxin A in 7.4% (2/27) of the isolates. Toxin production was detected only in *S. aureus*, and none of the isolates produced more than one toxin.

Listeria spp. in raw bulk milk

Listeria spp. was found in raw bulk milk from 13 farms (5.9%), and the number of *Listeria* spp. ranged between 20 and 1×10^3 cfu/mL. By 16S rRNA gene sequencing, *L. monocytogenes*, *L. innocua*, *L. seelingeri*, and *L. grayi* were found in 6, 4, 2, and 1 farms, respectively. Using a set of tests including Gram staining (positive), oxidase test (negative), catalase test (negative), and hemolysis (positive), the presence of *L. monocytogenes* was confirmed in milk from six farms (2.7%). This bacterium was found in farms from four different municipalities: Gjakova (n = 2), Lipjan (n = 2), Rahovec (n = 1), and Sharr (n = 1) (Supplementary Table 1). The three virulence genes *prfA*, *actA*, and *inlA* are quite common in *L. monocytogenes* [31]. As expected, the presence of these three genes in the six *L. monocytogenes* isolates from the respective farms was found (Table 3).

Discussion

Raw milk represents an important part of nutrition in Kosovo, but little is known about the microbiological quality of raw bulk milk, especially with regard to contamination and the prevalence of foodborne pathogens. To investigate this aspect, milk samples from 221 farms across the country were collected and analyzed. Results showed that only 6.8% (with total cell numbers < 10^5 cfu/mL) of the samples complied with the EU regulations of the microbial quality of milk [32]. The MAFRED regulations are less strict, with a TVC of 5 x 10^5cfu/mL as the upper limit [33]; however, only 43% of the samples (95/221) were acceptable within this national standard [33].

From each farm, samples were collected every second month at three time points. This sampling regime allowed us to assess the general microbial quality of a farm over time and partly during changing seasons (winter to summer). No significant variations in the microbial quality of milk was observed, but a significant variation in microbial quality between farms, irrespective of geographical location, was recorded. This indicates that contamination was most likely not related to the regions or season but rather to the milk production facilities at the individual farms (Supplementary Table 1). We found 43% of the farms (95/221) satisfied the MAFRED standard with regard to TVC limit (< 5×10^5 cfu/mL). Interestingly, in a previous study in 2007 from Kosovo, a large number of samples (from 364 farms) of bulk tank milk were collected, and 80 of these farms (20.6%) were found with TVCs below 5×10^5 cfu/mL [35]. Thus, these studies indicate that some improvement in milk quality occurred in recent years. Another study from 2008 on the microbial quality of raw milk in Macedonia, a neighboring country of Kosovo, revealed that 41.45% of raw milk had TVCs below 4×10^5 cfu/mL, which is the upper acceptance level of TVC in milk in Macedonia [33]. Using this limit (4×10^5 cfu/mL), only about 34% of farms in Kosovo have acceptable TVCs, suggesting that the current raw milk quality in Kosovo is inferior to milk in Macedonia in 2008. In addition, in different countries in Europe, the microbiological quality of raw milk is much better than in developing countries such as Kosovo [20,36]. It is well documented that several factors can influence the microbial quality of raw milk, including hygienic conditions of the farms and feed and herd size [12-16]. The farms in the regions Ferizaj, Gjakova, and Rahovec were shown to have highest level of TVCs compared to farms in other regions (Supplementary Table 1). This means that measurements such as more and stricter inspection and better management practices need to be implemented in these regions. Sraiiri *et al*. [37] showed that monitoring of herd management practices may allow a better indirect control of raw milk management than any of other factors, such as increasing the number of sampled cows or increasing the frequency of microbiological analyses of the raw milk. Similarly, another report showed that frequent microbiological analyses of raw milk and sharing the results with farmers can increase their hygiene awareness, which will eventually lead to better farming practices, thereby improving the microbial quality of milk [38]. Another factor that affects the quality of raw milk is herd size [12,15]. Our results showed that there were significant differences between the farms with large herd size (> 10 cows) and the farms with small hard size (< 5 cows) (Figure 2), which is in line with the results from a previous study where the quality of milk was shown to be better in large herds than in small ones [36].

The high number of TVCs found in raw bulk milk might indicate the presence of a great diversity of microorganisms, some of which can be foodborne pathogens such us *Salmonella*, *Listeria*, *Staphylococcus*, or *Escherichia coli*. This study conducted an analysis of the presence of two different foodborne pathogens, *L. monocytogenes* and *S. aureus*.

It was found that *S. aureus* was present in milk samples in 39.8% of the farms examined, and in 36 farms (16.3%), the counts of *S. aureus* were more than 10^5 cfu/mL. The presence of *S. aureus* in milk can originate from fecal contamination of the udder or milk equipment [39]. In recent years, it has been reported that the prevalence of *S. aureus* in raw bulk milk in EU varied between 12% and 75% [29]. In Balkan countries, few studies have been reported. The prevalence of this bacterium was 18% and 32% in farms in Albania and Bosnia and Herzegovina, respectively [4,30]. Hence, our results indicate that prevalence of *S. aureus* in Kosovo is higher compared to Albania but not to Bosnia and Herzegovina.

One of the causative agents of foodborne intoxication is *Staphylococcus* spp. toxin production, with *S. aureus* being the most prominent species [28]. Based on the food safety standards, *Staphylococcus* spp., and particularly *S. aureus* at high numbers (> 10^5 cfu/mL), can produce toxins that severely affect consumers [40]. At higher cell numbers, *S. aureus* can trigger toxin production (A, B, C, D, and E). *S. aureus* in our study was present at high cell levels (> 10^5 cfu/mL) in 30.7% of bulk tank milk samples tested. Several countries have reported that outbreaks of staphylococcal food poisoning are related to SEA, SEB, SEC, and SED enterotoxin [29]. In Bosnia and Herzegovina, it has been reported that 43% of the isolated *S. aureus* from milk carry one or more genes encoding staphylococcal toxins with highest prevalence of SEB, SEC, and SED toxins, a result that is comparable to that in our study [4].

L. monocytogenes is reported to be present in poorly preserved silage and/or in poor hygiene conditions in farms that are the common source of infection in dairy cows [41,42]. Different species of *Listeria* exist, but only *L. monocytogenes* and *L. ivanovii* have been reported to be the major pathogens in humans and animals, respectively. Cleaning procedure is not sufficient to eliminate *L. monocytogenes* [43]. Based on our results, the presence of *L. monocytogenes* in bulk tank milk was low (2.7%). Previous reports have shown that the prevalence of *L. monocytogenes* varied between 2.5% and 6% in bulk tank milk in Europe [43,44], while in one study from New York, *L. monocytogenes* was identified in 12.6% of dairy herds [45]. Consumption of such unpasteurized and contaminated raw bulk milk or processed milk would pose a potential health risk for consumers due to the high cell concentrations (up to 10^3 cfu/mL). It is well documented that production of cheese (particularly soft cheeses and fresh cheeses) or other milk-based products from unpasteurized milk infected with *L. monocytogenes* might increase the risk of infection [46]. In Kosovo, some of the traditional cheeses are made from unpasteurized milk.

Notably, regional differences in the prevalence of *S. aureus* and *L. monocytogenes* were not observed (Supplementary Table 1). However, differences were observed on the farm level rather than regional level, which might indicate differences in individual milking or hygiene practices. No obvious correlation between total counts and contamination with *S. aureus* and/or *L. monocytogenes* was found (Supplementary Table 1). This indicates that raw bulk milk is a good reservoir of these two foodborne pathogens, regardless the presence or absence of other bacteria in the same milk. However, *S. aureus* and *L. monocytogenes* were mostly present in raw milk collected from farms with a herd size smaller than eight cows. This indicates that these two foodborne pathogens are present more frequently in farms with small herds than in farms with large herds.

Conclusions

The present results indicate that the hygienic quality of bulk tank milk in Kosovo is poor and needs to be improved. Potentially enterotoxigenic *S. aureus* and virulent *L. monocytogenes* are occasionally found in Kosovo farms. There is a need to increase the hygiene in dairy farms, and some measures should be directed to improve hygienic conditions during the milking process and milk transport. Possible reasons for the presence of high total viable count contamination include lack of adequate cleaning and improper milking disinfection equipment and suboptimal milk storage and transport. One of the important issues in milk production in Kosovo dealing with poor microbiological quality of milk, which can be overcome by improving the sanitation in the farms. This can be accomplished by providing more information to farmers about the relationship between hygiene and milk quality and also through relevant and practical training given by veterinary and health authorities. Increased herd size and more organized and professional farming practices will probably improve the microbiological quality of raw milk as well.

Acknowledgements

This study was supported by a grant from Ministry for Foreign Affairs (number 332160UW) under the Program for Higher Education, Research and Development 2010-2014 Norway (HERD) and the Royal Norwegian Embassy of Kosovo. We thank the 221 dairy farms for their participation in the study. Thanks to Linda Godager for excellent technical assistance.

Authors' contributions

IM made the major contribution on this article in all aspects from the design of the study, all analytical work, statistics, and writing of the manuscript. HB and SM participated on design of the study, sample collection, identification of farms, and helped in manuscript improvement with his comments and discussions. IFN and DBD critically contributed to design of the study, supervision of analytical work, interpretation of results, and helped in manuscript improvement with their scientific comments. All authors read and approved the final manuscript.

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Conflict of interests: No conflict of interests is declared.

Annex – Supplementary items

Supplementary Table 1. Results for total viable counts (cfu/mL), *Staphylococcus* spp. and *Listeria* spp. in raw bulk milk.

Total	Farm code	Municipality	Total count (CFU/mL)	*S. aureus*	*L. monocytogenes*
1	61	Decan	15×10^3	38×10^1	0
2	67	Decan	10×10^4	39×10^1	0
3	69	Decan	22×10^4	37×10^2	0
4	56	Decan	23×10^4	14×10^2	0
5	64	Decan	23×10^4	29×10^2	0
6	68	Decan	31×10^4	66×10^2	0
7	65	Decan	31×10^4	19×10^2	0
8	59	Decan	56×10^4	32×10^2	0
9	63	Decan	71×10^4	34×10^3 [a]	0
10	57	Decan	72×10^4	10×10^2	0
11	70	Decan	82×10^4	13×10^2	0
12	66	Decan	87×10^4	12×10^2	0
13	60	Decan	95×10^4	19×10^2	0
14	58	Decan	20×10^5	14×10^2	0
15	62	Decan	21×10^5	13×10^2	0
16	116	Ferizaj	28×10^3	34×10^1	0
17	126	Ferizaj	14×10^4	13×10^2	0
18	108	Ferizaj	16×10^4	17×10^2	0
19	117	Ferizaj	19×10^4	18×10^2	0
20	115	Ferizaj	21×10^4	28×10^2	0
21	125	Ferizaj	26×10^4	18×10^2	0
22	127	Ferizaj	31×10^4	19×10^2	0
23	121	Ferizaj	32×10^4	19×10^2	0
24	114	Ferizaj	35×10^4	14×10^2	0
25	132	Ferizaj	35×10^4	112×10^2 [c]	0
26	118	Ferizaj	42×10^4	94×10^2	0
27	110	Ferizaj	49×10^4	19×10^2	0
28	120	Ferizaj	53×10^4	89×10^2	0
29	122	Ferizaj	59×10^4	12×10^2	0
30	119	Ferizaj	71×10^4	17×10^3 [a]	0
31	109	Ferizaj	89×10^4	13×10^2	0
32	131	Ferizaj	93×10^4	124×10^3 [a]	0
33	123	Ferizaj	13×10^5	13×10^2	0
34	124	Ferizaj	15×10^5	21×10^3 [a]	0
35	130	Ferizaj	16×10^5	20×10^2	0
36	128	Ferizaj	18×10^5	54×10^2	0
37	111	Ferizaj	23×10^5	76×10^2	0
38	129	Ferizaj	26×10^5	14×10^2	0
39	113	Ferizaj	26×10^5	66×10^2	0
40	112	Ferizaj	27×10^5	3×10^2	0
41	133	Ferizaj	31×10^5	62×10^4 [a]	1×10^3 [e]
42	202	Gjakova	70×10^3	32×10^1	0
43	194	Gjakova	16×10^4	29×10^2	0
44	207	Gjakova	21×10^4	39×10^2	0
45	204	Gjakova	53×10^4	86×10^2	5×10^1 [g]
46	206	Gjakova	54×10^4	19×10^2	0
47	198	Gjakova	62×10^4	14×10^2	0
48	196	Gjakova	62×10^4	14×10^2	0
49	208	Gjakova	86×10^4	12×10^2	0
50	197	Gjakova	23×10^5	96×10^2	3×10^2 [d]
51	195	Gjakova	28×10^5	19×10^2	0
52	203	Gjakova	32×10^5	13×10^2	0
53	200	Gjakova	36×10^5	11×10^4 [a,c]	0
54	199	Gjakova	39×10^5	13×10^2	0
55	201	Gjakova	52×10^5	89×10^2	2×10^1 [d]
56	205	Gjakova	57×10^5	87×10^2	0
57	89	Istog	54×10^3	12×10^1	0

Total	Farm code	Municipality	Total count (CFU/mL)	*S. aureus*	*L. monocytogenes*
58	87	Istog	74×10^3	21×10^1	0
59	92	Istog	12×10^4	10×10^2	0
60	100	Istog	14×10^4	42×10^1	0
61	99	Istog	15×10^4	19×10^2	0
62	88	Istog	16×10^4	12×10^2	0
63	90	Istog	21×10^4	18×10^2	0
64	105	Istog	22×10^4	18×10^2	0
65	98	Istog	23×10^4	18×10^2	0
66	103	Istog	26×10^4	19×10^2	0
67	106	Istog	35×10^4	84×10^2	0
68	107	Istog	39×10^4	122×10^2 [a]	0
69	96	Istog	42×10^4	1×10^2	0
70	93	Istog	44×10^4	3×10^2	0
71	101	Istog	54×10^4	89×10^2	0
72	102	Istog	57×10^4	96×10^2	0
73	104	Istog	81×10^4	62×10^3 [a]	0
74	95	Istog	84×10^4	10×10^2	0
75	91	Istog	17×10^5	19×10^2	0
76	97	Istog	20×10^5	17×10^2	0
77	94	Istog	30×10^5	12×10^3 [a]	0
78	41	Lipjan	86×10^3	34×10^1	0
79	36	Lipjan	93×10^3	10×10^2	0
80	40	Lipjan	15×10^4	12×10^2	0
81	55	Lipjan	16×10^4	19×10^2	0
82	50	Lipjan	26×10^4	19×10^2	0
83	44	Lipjan	26×10^4	43×10^2	0
84	51	Lipjan	30×10^4	19×10^2	0
85	54	Lipjan	33×10^4	25×10^2	0
86	43	Lipjan	43×10^4	3×10^2	0
87	47	Lipjan	47×10^4	224×10^2 [a]	0
88	42	Lipjan	49×10^4	40×10^2	0
89	48	Lipjan	49×10^4	13×10^2	0
90	39	Lipjan	61×10^4	13×10^2	0
91	53	Lipjan	68×10^4	28×10^2	0
92	37	Lipjan	71×10^4	12×10^2	0
93	45	Lipjan	72×10^4	19×10^2	0
94	52	Lipjan	86×10^4	18×10^2	0
95	49	Lipjan	95×10^4	21×10^2	4×10^2 [d]
96	38	Lipjan	13×10^5	18×10^2	0
97	46	Lipjan	14×10^5	12×10^3 [a]	6×10^1 [d]
98	28	Podujeva	4×10^4	36×10^1	0
99	22	Podujeva	12×10^4	21×10^1	0
100	33	Podujeva	15×10^4	11×10^2	0
101	35	Podujeva	17×10^4	26×10^2	0
102	21	Podujeva	2×10^5	19×10^2	0
103	31	Podujeva	22×10^4	19×10^2	0
104	26	Podujeva	27×10^4	19×10^2	0
105	32	Podujeva	31×10^4	83×10^2	0
106	27	Podujeva	37×10^4	136×10^2 [b]	0
107	24	Podujeva	41×10^4	19×10^2	0
108	34	Podujeva	49×10^4	112×10^2 [a]	0
109	29	Podujeva	48×10^4	32×10^3 [a]	0
110	25	Podujeva	49×10^4	17×10^1	0
111	30	Podujeva	57×10^4	73×10^2	0
112	23	Podujeva	58×10^4	46×10^3 [a]	0
113	136	Rahovec	27×10^3	16×10^1	0
114	143	Rahovec	40×10^3	32×10^1	0
115	157	Rahovec	50×10^3	13×10^1	0
116	138	Rahovec	87×10^3	12×10^1	0
117	191	Rahovec	90×10^3	12×10^2	0

Total	Farm code	Municipality	Total count (CFU/mL)	*S. aureus*	*L. monocytogenes*
118	140	Rahovec	12×10^4	12×10^2	0
119	192	Rahovec	12×10^4	40×10^1	0
120	141	Rahovec	13×10^4	32×10^1	0
121	147	Rahovec	16×10^4	16×10^2	0
122	173	Rahovec	21×10^4	13×10^2	0
123	193	Rahovec	21×10^4	33×10^2	0
124	174	Rahovec	23×10^4	47×10^2	0
125	190	Rahovec	30×10^4	18×10^2	0
126	158	Rahovec	36×10^4	19×10^1	0
127	179	Rahovec	38×10^4	1×10^2	0
128	186	Rahovec	54×10^4	7600	0
129	178	Rahovec	66×10^4	43×10^2	0
130	176	Rahovec	88×10^4	12×10^2	2×10^2 [d]
131	159	Rahovec	92×10^4	13×10^2	0
132	162	Rahovec	12×10^5	19×10^2	0
133	150	Rahovec	13×10^5	12×10^2	0
134	171	Rahovec	13×10^5	11×10^2	0
135	148	Rahovec	14×10^5	75×10^2	0
136	164	Rahovec	15×10^5	4×10^2	0
137	139	Rahovec	15×10^5	62×10^4 [a]	0
138	188	Rahovec	16×10^5	81×10^2	0
139	134	Rahovec	17×10^5	12×10^2	0
140	180	Rahovec	17×10^5	21×10^2	0
141	183	Rahovec	17×10^5	20×10^2	0
142	181	Rahovec	17×10^5	14×10^2	0
143	145	Rahovec	17×10^5	12×10^4 [a]	0
144	142	Rahovec	18×10^5	11×10^4 [a,b]	2×10^2 [e]
145	182	Rahovec	18×10^5	19×10^2	0
146	163	Rahovec	18×10^5	52×10^2	0
147	184	Rahovec	19×10^5	19×10^2	0
148	135	Rahovec	19×10^5	54×10^2	0
149	172	Rahovec	21×10^5	19×10^2	0
150	189	Rahovec	23×10^5	89×10^2	0
151	137	Rahovec	24×10^5	28×10^3 [a]	0
152	167	Rahovec	25×10^5	19×10^2	0
153	146	Rahovec	26×10^5	76×10^2	0
154	165	Rahovec	27×10^5	14×10^2	0
155	156	Rahovec	28×10^5	18×10^2	0
156	166	Rahovec	30×10^5	13×10^2	0
157	160	Rahovec	35×10^5	72×10^2	0
158	170	Rahovec	35×10^5	12×10^4 [a]	0
159	153	Rahovec	36×10^5	18×10^2	0
160	149	Rahovec	38×10^5	23×10^3 [a]	0
161	185	Rahovec	39×10^5	18×10^2	0
162	144	Rahovec	42×10^5	13×10^2	0
163	154	Rahovec	46×10^5	98×10^2	0
164	155	Rahovec	50×10^5	17×10^2	0
165	177	Rahovec	56×10^5	23×10^3 [a]	0
166	151	Rahovec	60×10^5	98×10^2	0
167	161	Rahovec	62×10^5	11×10^4 [a]	4×10^1 [e]
168	187	Rahovec	67×10^5	13×10^2	0
169	152	Rahovec	68×10^5	17×10^2	0
170	175	Rahovec	72×10^5	11×10^3 [a,]	8×10^2 [e]
171	168	Rahovec	86×10^5	12×10^3 [a]	0
172	169	Rahovec	99×10^5	12×10^4 [a]	0
173	83	Sharr	19×10^3	16×10^1	0
174	76	Sharr	14×10^4	12×10^2	0
175	72	Sharr	18×10^4	17×10^2	0
176	86	Sharr	23×10^4	19×10^2	0
177	79	Sharr	45×10^4	3×10^2	0

Total	Farm code	Municipality	Total count (CFU/mL)	*S. aureus*	*L. monocytogenes*
178	80	Sharr	48×10^4	18×10^2	0
179	78	Sharr	49×10^4	2×10^2	0
180	77	Sharr	49×10^4	22×10^3 [a]	0
181	84	Sharr	68×10^4	39×10^1	8×10^2 [d]
182	82	Sharr	68×10^4	13×10^2	0
183	74	Sharr	76×10^4	20×10^2	0
184	85	Sharr	77×10^4	19×10^2	0
185	73	Sharr	82×10^4	31×10^3 [a]	0
186	75	Sharr	92×10^4	20×10^2	0
187	71	Sharr	97×10^4	16×10^4 [a,]	0
188	81	Sharr	98×10^4	14×10^2	0
189	6	Skenderaj	46×10^3	42×10^1	0
190	2	Skenderaj	10×10^4	92×10^1	0
191	1	Skenderaj	16×10^4	20×10^2	0
192	7	Skenderaj	23×10^4	33×10^2	0
193	9	Skenderaj	31×10^4	39×10^2	0
194	12	Skenderaj	46×10^4	132×10^2 [a]	0
195	16	Skenderaj	49×10^4	47×10^1	0
196	13	Skenderaj	52×10^4	21×10^3 [a]	0
197	11	Skenderaj	52×10^4	23×10^3 [a]	0
198	14	Skenderaj	67×10^4	10×10^3 [a]	0
199	10	Skenderaj	67×10^4	63×10^2	0
200	5	Skenderaj	70×10^4	11×10^2	0
201	3	Skenderaj	72×10^4	13×10^2	0
202	8	Skenderaj	79×10^4	82×10^3 [a]	0
203	18	Skenderaj	92×10^4	2×10^2	0
204	4	Skenderaj	94×10^4	89×10^2	0
205	17	Skenderaj	11×10^5	24×10^2	0
206	19	Skenderaj	16×10^5	51×10^3 [a]	0
207	15	Skenderaj	17×10^5	98×10^2	2×10^1 [f]
208	20	Skenderaj	17×10^5	98×10^2	0
209	213	Suhareka	27×10^4	19×10^2	0
210	220	Theranda	33×10^4	19×10^2	0
211	215	Theranda	46×10^4	18×10^2	0
212	216	Theranda	48×10^4	18×10^2	0
213	209	Theranda	52×10^4	21×10^2	0
214	214	Theranda	59×10^4	28×10^3 [a]	0
215	211	Theranda	62×10^4	13×10^2	0
216	221	Theranda	71×10^4	20×10^2	0
217	212	Theranda	72×10^4	69×10^2	1×10^3 [f]
218	219	Theranda	87×10^4	13×10^3 [a]	0
219	210	Theranda	87×10^4	13×10^2	0
220	218	Theranda	96×10^4	13×10^2	0
221	217	Theranda	97×10^4	94×10^2	0

[a] *S. aureus*; [b] *S. infantarius*; [c] *S. simmulans*; [d] *L. monocytogenes*; [e] *L. innocua*; [f] *L. seelingeri;* [g] *L. grayi;* N: not analyzed