Thesis of doctoral (Ph.D.) dissertation

MICROBIOLOGICAL CHARACTERIZATION OF UNPASTEURIZED SHEEP MILK

Andualem Tonamo Tema

Ph.D. candidate

Supervisors: Prof. Dr. István Komlósi, *D.Sc.*

professor

Dr. Ferenc Árpád Peles, *Ph.D.*

assistant professor

UNIVERSITY OF DEBRECEN

Doctoral School of Animal Science

Debrecen, 2021

1. BACKGROUND AND AIMS OF THE DISSERTATION

Hungary has approximately 790,000 heads of ewes out of 1.1 million heads of ovine flock size in late 2019 (HUNGARIAN CENTRAL STATISTICAL OFFICE, 2019). The available data on FAOSTAT (2019) database indicated that the ovine milk production of Hungary in 2019 was 1,580 metric tons. There are currently 19 dairy sheep farms in Hungary where milking data are regularly recorded. Some of the dairy sheep breeds kept on these farms are Milking Tsigai, Lacaune, British Milksheep, and East Friesian breeds and in few farms, Awassi and Merino breed has been milked (HUNGARIAN SHEEP and GOAT BREEDERS' ASSOCIATION, 2020).

At commercial dairy sheep farms, the key parameters of milk regularly inspected are total plate count, chemical composition (fat and protein content) and somatic cell count (PIRISI et al., 2007). Moreover, pH and the appearance of milk are also important (PARK et al., 2013). Understanding of public health and food safety concerns has led to more interest in milk hygiene in ovine to reduce risk and ensure optimum quality of consumption (GONZALO, 2017).

In Hungary, where sheep are milked, the consumption of milk and its products is so far less in terms of quantity and quality. It is therefore very important to expand dairy sheep farms with milk type ewes, with the aim of producing hygienic milk and its products. Respecting acceptable limit values for total plate count and other pathogenic microorganisms is another issue that needs consideration in ovine milk sector. Microbiological studies on ovine-associated products have so far been relatively neglected, although there is an upward worldwide interest in the consumption of ovine milk and milk products. This is also true for Hungary, where the microbiological quality of raw ovine milk and ovine cheese samples has not been fully investigated (KUKOVICS et al., 2009).

Basic data on the microbiological quality of ovine-associated samples (e.g. udder surface, raw milk, and cheese) for further research to improve the quality of ovine-originated foods are critical. Moreover, studying the virulence factor and antibiotic resistance of pathogenic bacterial strains such as *S. aureus* is important from a public health point of view. To the best of our knowledge, this is the first report evaluating the bacterial count and characterizing bacterial strains of ovine udder surface samples in Hungary. The following specific objectives have been pursued through the duration of the research.

- Examination of the microbiological status (total plate count, *Enterobacteriaceae* count, *Escherichia coli* count, and *Staphylococcus aureus* count) of udder surface samples.
- Examination of the microbiological status (total plate count, *Enterobacteriaceae* count, *Escherichia coli* count, *Staphylococcus aureus* count, and lactic acid bacteria count) and California Mastitis Test of individual raw milk samples.
- Examination of the microbiological status (total plate count, *Enterobacteriaceae* count, *Escherichia coli* count, *Staphylococcus aureus* count, psychrotrophic bacteria count, and lactic acid bacteria count) of bulk tank milk samples.
- Examination of the microbiological status (*Enterobacteriaceae* count, *Escherichia coli* count, *Staphylococcus aureus* count, and lactic acid bacteria count) of cheese samples.
- Determination of the coefficient of correlation between the major bacterial counts; i.e. total plate count and *Enterobacteriaceae* count of corresponding udder surface and individual raw milk samples.
- Analysis of microbiota community in some raw milk samples.
- Phenotypic (tellurite production, lecithinase activity, coagulase test, hemolysis, catalase test, oxidase test and antibiotic resistance) and genotypic (enterotoxin gene presence) characterizations of staphylococci strains.
- Phenotypic (catalase test, oxidase test, and antibiotic resistance) properties of lactic acid bacteria isolates.

2. MATERIALS AND METHODS

In this study, four sheep farms located in Hajdú-Bihar County (Farm I, II, and III) and Jász-Nagykun-Szolnok County (Farm IV) of eastern Hungary were enrolled. One hundred seventy-three (77 udder surface, 86 individual raw milk, and 10 bulk tank milk) samples of five ewe breeds were examined for bacteriological quality. In addition, 15 cheese samples that were purchased from three sources (9 from the 79th OMÉK, 3 from the market in Debrecen, and 3 from the farmer near to Debrecen) were tested for bacteriological quality. Data on flock size, breed, feeding, housing, frequency, and time of milking were gathered during farm visits for sampling.

Ewes that had a detectable mammary gland abnormality and clinical mastitis were excluded. Among ewes with clinically healthy udder, five to twenty-one animals with mixed-parity were randomly selected for the udder surface and individual raw milk sampling. Milk sampling was performed in compliance with the guidelines of the International Dairy Federation for milk and milk products sampling **(ISO 707:2008)**. California Mastitis Test was performed at the farm by mixing 2-3 mL of udder half milk samples with the same amount of reagents. On the same day as the collection of the samples, bacteriological analysis was carried out immediately in the microbiological laboratory of the Institute of Food Sciences at the University of Debrecen. The bacterial counts that were examined in our study were shown in Table 1.

Table 1: Procedure detail for the bacterial count

Bacterial count	**Culture media**	**Incubation**	**Standard**
Total plate count	Plate count agar	30 ^oC, 72 hrs	ISO 4833-1:2013
Enterobacteriaceae	Violet Red Bile Glucose agar	37 ^oC, 24 hrs	ISO 21528-2:2017
Escherichia coli	Tryptone Bile X-Glucoronide agar	37 ^oC, 24 hrs	ISO 16649-2:2001
Staphylococcus aureus	Baird-Parker agar	37 ^oC, 48 hrs	ISO 6888-1:2008
Lactic acid bacteria	de Man, Rogosa and Sharpe agar	30 ^oC, 72 hrs	ISO 15214:2005
Psychrotrophic bacteria	Plate count agar	7 ^oC, ten days	ISO 17410:2005

Furthermore, five ovine raw milk samples were analysed for microbiota composition. Three milk samples were analysed in the Eurofins Genomics GmbH, Germany for microbiota profiling. The remaining two milk samples were analysed for microbiota diversity at Biomi Ltd. Company, Hungary. The former one (Eurofins Genomics) was by 16S rRNA gene sequencing method by targeting the V3-V4 gene region and the latter one (Biomi Ltd.) was by LoopSeq™ 16S-18S Microbiome SSC 24-Plex Kit (Version 1.7, July 2019) method (https://www.loopgenomics.com/16s) with single sample calibration.

A total of 45 staphylococci and 11 lactic acid bacteria isolates identified either by MALDI-TOF MS Biotyper (KAČÁNIOVÁ et al., 2020) and/or API Staph (PEXARA et al., 2016) and/or 16S rRNA genome sequencing (LANE, 1991) were characterized. Characterizations were carried out for all colonies, which were catalase and oxidase tests, while coagulase and hemolysis tests were carried out for only staphylococcal isolates. Staphylococci and lactic acid bacterial strains were tested against 8 and 10 antibiotics (Biolab Ltd., Hungary) (Table 2) for antibiotic resistance respectively in the microbiological laboratory of the Institute of Food Sciences at the University of Debrecen. The disk diffusion method has been applied to Mueller-Hinton (staphylococci isolates) and MRS (lactic acid bacteria isolates) agar (Biolab Ltd., Hungary) in compliance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2017).

Table 2: Antibiotics used for antibiotic resistance test

Antibiotics*	**Dosages**
Penicillin G (P)	10 U/disk
Cefoxitin (FOX)	30 µg/disk
Chloramphenicol (C)	30 µg/disk
Clindamycin (DA)	2 µg/disk
Erythromycin (E)	15 µg/disk
Gentamicin (CN)	10 µg/disk
Tetracycline (TE)	30 µg/disk
Trimethoprim/Sulfamethoxazole (SXT)	1.25 + 23.75 µg/disk
Streptomycin (S)	10 µg/disk
Vancomycin (VA)	30 µg/disk

**Staphylococcus* strains were evaluated against the first eight antibiotics (penicillin G to trimethoprim/sulfamethoxazole), and lactic acid bacteria strains were against all ten antibiotics (penicillin G to vancomycin).

45 staphylococci strains were evaluated for the presence of 13 enterotoxin genes. DNA was extracted from staphylococci strains using the PrepManTM Ultra Sample Preparation Reagent (Biocenter Kft., Hungary) as urged by the manufacturer. To detect the well-characterized classical five SEs (SEA, SEB, SEC, SED and SEE), the multiplex PCR reaction was carried out. Furthermore, three duplex (SEG and SEI, SEH and SER, SE*l*M and SE*l*O) and two uniplex (SEJ, SE*l*N) PCR reactions were to detect 5 new forms of SEs (SEG, SEH, SEI, SEJ, and SER) and 3 SE*l*s (SE*l*M, SE*l*N, and SE*l*O). Amplification cycles were programmed as 5 min at 95 °C for initial denaturation; 35 cycle, 30 sec at 95 °C for denaturation; 30 s at 52 °C for annealing; 1 min elongation at 72°C; and 10 min final elongation step at 72 °C.

One percent gel was prepared with 80 mL of 1 × TBE buffer containing ethidium bromide and 0.1 g agarose (Lab Mark Ltd., Czech Republic). Four µL of MidoriGreen Advance stain (Nucleotest Bio Kft., Hungary) was dissolved in the liquid gel before pouring into a gel tray. After the gel was dried for 30 minutes, it was placed into a gel box. Electrophoresis was carried out at 120 V for 40 minutes for all PCR products. For molecular weight marker, 100 bp GeneRuler™ Plus DNA Ladder (Biocenter Kft., Hungary) was used. The ethidium bromide was used to stain the gels and photographed under FluorChem M system (Bio-Science Kft., Hungary).

Positive reference staphylococci strains used for the PRC reaction were shown in Table 3. Using the primers shown in Table 4, the detection was carried out in the microbiological laboratory of the Institute of Food Sciences at the University of Debrecen. For PCR, the procedures were followed as suggested by MEHROTRA et al. (2000), SHARMA et al. (2000), BANIA et al. (2006), and CHIANG et al. (2008) with a minor change.

Table 3: The positive reference staphylococci strains used for the PCR reaction

Strains ID	**Staphylococcal enterotoxin produced**
S. aureus ATCC29213	SEA, SEG, SEI
ATCC14458	SEB
ATCC19095	SEC, SEG, SEH, SEI
ATCC23235	SED, SEG, SEI, SEJ, SER
ATCC27664	SEE
SA54A	SE*l*M, SE*l*O
SA54B	SE*l*N

Table 4: PCR primers used for staphylococcal enterotoxins detection

Genes	Primers	Sequence (5´- 3´)	Size	Amplicon size (bp)	References
sea	GSEAR-F	GGTTATCAATGTGCGGGTGG	20	102	**MEHROTRA et al. (2000)**
	GSEAR-R	CGGCACTTTTTTCTCTTCGG	20		
seb	GSEBR-F	GTATGGTGGTGTAACTGAGC	20	164	
	GSEBR-R	CCAAATAGTGACGAGTTAGG	20		
sec	GSECR-F	AGATGAAGTAGTTGATGTGTATGG	24	451	
	GSECR-R	CACACTTTTAGAATCAACCG	20		
sed	GSEDR-F	CCAATAATAGGAGAAAATAAAAG	23	278	
	GSEDR-R	ATTGGTATTTTTTTTCGTTC	20		
see	SA-U	TGTATGTATGGAGGTGTAAC	20	213	**SHARMA et al. (2000)**
	SA-E rev	GCCAAAGCTGTCTGAG	16		
seg	SEG-F	GTTAGAGGAGGTTTTATG	18	198	**BANIA et al. (2006)**
	SEG-R	TTCCTTCAACAGGTGGAGA	19		
seh	SEH-F	CAACTGCTGATTTAGCTCAG	20	173	
	SEH-R	CCCAAACATTAGCACCA	17		
sei	SEI-F	GGCCACTTTATCAGGACA	18	328	
	SEI-R	AACTTACAGGCAGTCCA	17		
sej	SEJ-F	GTTCTGGTGGTAAACCA	17	131	
	SEJ-R	GCGGAACAACAGTTCTGA	18		

Genes	Primers	Sequence (5´- 3´)	Size	Amplicon size (bp)	References
selm	SEM-F	CATATCGCAACCGCTGA	17	148	
	SEM-R	TCAGCTGTTACTGTCGA	17		
seln	SEN-F	GGCAATTAGACGAGTCA	17	237	
	SEN-R	ATCGTAACTCCTCCGTA	17		
selo	SEO-F	GTCAAGTGTAGACCCTA	17	288	
	SEO-R	TGTACAGGCAGTATCCA	17		
selp	SEP-F	TCAAAAGACACCGCCAA	17	396	
	SEP-R	ATTGTCCTTGAGCACCA	17		
ser	SER1-F	AGATGTGTTTGGAATACCCTAT	22	123	**CHIANG et al. (2008)**
	SER2-R	CTATCAGCTGTGGAGTGCAT	20		

2.1. Data analysis

Data were analysed by GraphPad Prism 3.02 (San Diego, California, USA) and SPSS (2019) version 26.0 (IBM SPSS Corp., Armonk, NY, USA). The effects on bacterial counts by farms (Farm I-IV), breed (Farm I), and year (2018 and 2019) were evaluated by one-way ANOVA, t-test (for two levels of factors), and the non-parametric Kruskal-Wallis test. The mean values were expressed by CFU/cm^2, CFU/mL, and CFU/g for udder surface, milk and cheese samples respectively. The Pearson correlation was used to evaluate the correlation between the major bacterial counts. The level of significance was considered at $P < 0.05$.

3. MAIN RESULTS

3.1. California Mastitis Test of udder halves milk

In this study, 124 udder halves-ovine milk samples were tested for California Mastitis Test (CMT). Of 124 udder halves milk samples, 16 (12.7%) were found to be weak positive-to-positive CMT. Out of 16 CMT weak positive-to-positive milk samples, eleven (68.8%) and five (31.2%) were recorded as weak positive (+) and positive (++) respectively (Table 5). Almost all individual raw milk samples with weak positive or positive CMT scores had a slightly higher TPC value than milk samples with negative CMT scores in all farms.

Table 5: Distribution of California Mastitis Test scores

Farm	**No. of udder halves milk sample examined**	**California Mastitis Test score**			
		Negative (-)	Weak positive (+)	Positive (++)	Overall (+ and ++)
Farm I	54	48 (88.9%)	5 (9.3%)	1 (1.8%)	6 (11.1%)
Farm II	30	27 (90.0%)	1 (3.3%)	2 (6.7%)	3 (10.0%)
Farm III	20	15 (75.0%)	3 (15.0%)	2 (10.0%)	5 (25.0%)
Farm IV	20	18 (90.0%)	2 (10.0%)	0 (0.0%)	2 (10.0%)
Overall	**124**	**108 (87.1%)**	**11 (8.9%)**	**5 (4.0%)**	**16 (12.9%)**

3.2. Total plate count of udder surface and milk samples

Total plate count (TPC) of udder surface (US) samples was ranged from 1.0 to 5.1 lg CFU/cm^2 with overall mean value of 2.5 ± 0.8 lg CFU/cm^2 (Table 6). With regard to individual raw milk (IRM) samples, TPC was between 1.0 and 5.5 lg CFU/mL with overall average value of 3.1 ± 1.1 lg CFU/mL. Bulk tank milk (BTM) samples had TPC values ranged from 5.2 to 8.9 lg CFU/mL with overall mean value of 7.0 ± 0.9 lg CFU/mL (Table 6).

Table 6: Total plate count in udder surface and milk samples (between 2018 and 2020)

Type of samples	**Selected statistical value**	**Farm I (n = 42)**	**Farm II (n = 15)**	**Farm III (n = 10)**	**Farm IV (n = 10)**	**Overall (n = 77)**
Udder surface ($lg CFU/cm^2$)	Minimum value	1.1	1.0	1.2	1.6	1.0
	Maximum value	5.1	4.1	3.2	3.6	5.1
	Mean ± SD	2.7 ± 1.0[a]	2.5 ± 0.7[ab]	2.2 ± 0.5[b]	2.4 ± 0.4[ab]	2.5 ± 0.8
Individual raw milk (lg CFU/mL)[*]		**Farm I (n = 51)**	**Farm II (n = 15)**	**Farm III (n = 10)**	**Farm IV (n = 10)**	**Overall (n = 86)**
	Minimum value	1.0	1.3	1.5	1.0	1.0
	Maximum value	5.4	5.5	5.0	2.5	5.5
	Mean ± SD	3.1 ± 1.1[a]	3.3 ± 1.0[a]	3.5 ± 0.9[a]	1.8 ± 0.4[b]	3.1 ± 1.1
Bulk tank milk (lg CFU/mL)		**Farm I (n = 0)**	**Farm II (n = 6)**	**Farm III (n = 3)**	**Farm IV (n = 1)**	**Overall (n = 10)**
	Minimum value	No milking	6.4	5.5	5.2	5.2
	Maximum value	No milking	8.9	6.9	5.2	8.9
	Mean ± SD	No milking	7.4 ± 0.6[a]	6.3 ± 0.4[b]	5.2	7.0 ± 0.9

[*]Total plate count of individual milk samples with positive CMT scores was included in the analysis due to a non-significance between individual raw milk samples with negative CMT (n = 108) scores and negative to positive CMT scores (n = 124). During the sampling period, milking was started on Farm I. [ab]Mean values in the same row with different letters are significantly ($P < 0.05$) different.

3.3. *Enterobacteriaceae* count of udder surface, milk and cheese samples

Enterobacteriaceae count (EBC) of US samples was ranged from 0.1 to 2.6 lg CFU/cm^2 with an average value of 1.2 ± 0.6 lg CFU/cm^2 (Table 7). With regard to IRM samples, EBC was between 0.0 and 3.9 lg CFU/mL with overall mean value of 1.9 ± 0.8 lg CFU/mL. Bulk tank milk samples had EBC values ranged from 3.9 to 7.0 lg CFU/mL with overall mean value of 5.1 ± 0.9 lg CFU/mL (Table 7).

Table 7: *Enterobacteriaceae* count in udder surface and milk samples (between 2018 and 2020)

Type of samples	**Selected statistical value**	**Farm I (n = 42)**	**Farm II (n = 15)**	**Farm III (n = 10)**	**Farm IV (n = 10)**	**Overall (n = 77)**
Udder surface (lg CFU/cm^2)	Minimum value	0.3	0.1	0.9	0.2	0.1
	Maximum value	2.6	1.9	1.1	0.7	2.6
	Mean ± SD	1.4 ± 0.7^{a}	1.0 ± 0.5^{ab}	1.1 ± 0.1^{ab}	0.5 ± 0.3^{b}	1.2 ± 0.6
Individual raw milk (lg CFU/mL)*		**Farm I (n = 51)**	**Farm II (n = 15)**	**Farm III (n = 10)**	**Farm IV (n = 10)**	**Overall (n = 86)**
	Minimum value	0.7	1.1	0.0	0.0	0.0
	Maximum value	1.4	3.9	2.2	1.4	3.9
	Mean ± SD	1.1 ± 0.3^{a}	2.3 ± 0.9^{b}	2.2 ± 0.0^{ab}	1.4 ± 0.0^{ab}	1.9 ± 0.8
Bulk tank milk (lg CFU/mL)		**Farm I (n = 0)**	**Farm II (n = 6)**	**Farm III (n = 3)**	**Farm IV (n = 1)**	**Overall (n = 10)**
	Minimum value	No milking	3.9	4.3	3.9	3.9
	Maximum value	No milking	7.0	6.8	3.9	7.0
	Mean ± SD	No milking	5.1 ± 0.9^{a}	5.6 ± 0.9^{a}	3.9	5.1 ± 0.9

**Enterobacteriaceae* count of individual milk samples with positive CMT scores was included in the analysis due to a non-significance between individual raw milk samples with negative CMT (n = 108) scores and negative to positive CMT scores (n = 124). During the sampling period, animals were not milked on Farm I. [ab]Mean values in the same row with different letters are significantly ($P < 0.05$) different.

The mean of EBC was 5.5 ± 0.1, 5.5 ± 0.2, 4.3 ± 0.6 and 0.0 ± 0.0 lg CFU/g in chives-flavoured cheese (CFC), garlic-flavoured-smoked cheese (GFSC), cumin-flavoured-smoked cheese (CFSC) and garlic-flavoured cheese (GFC), respectively. There was significant difference ($P < 0.05$) between cheese samples in the case of EBC. EBC mean value in CFSC was significantly lower ($P < 0.05$) compared to CFC and GFSC.

3.4. *Escherichia coli* count of milk and cheese samples

E. coli was not detected in all US samples except for Farm II US samples, where *Escherichia coli* was not tested. *E. coli* was not tested for Farm II individual animal milk samples and interestingly, it was not present in IRM samples obtained from three other farms. In the case of BTM samples from Farm III and IV, the mean count of *E. coli* was 2.9 ± 0.5 and 3.8 lg CFU/mL, respectively. The mean value of ECC was 5.1 ± 0.4 and 4.4 ± 0.3 lg CFU/g in GFSC and CFC respectively, while not detected in CFSC and GFC samples. Higher count of *E. coli* was found in GFSC.

3.5. *Staphylococcus aureus* count in udder surface, milk and cheese samples

Out of 77 udder surfaces examined from four farms, *Staphylococcus aureus* was detected in only two samples (2.6%) with *S. aureus* count (SAC) of < 1 lg CFU/mL. Overall, out of 86 IRM samples examined, *S. aureus* was detected in six (7.0%) samples with overall mean value of 2.9 ± 0.6 lg CFU/mL. Out of 10 BTM samples, *S. aureus* was detected in only two (20%) samples with a mean of 3.4 ± 0.6 lg CFU/mL. Coagulase-positive staphylococci mean value was 5.7 ± 0.2 lg CFU/g in CFC and not detected in the other three cheese types. Significantly, a higher value ($P < 0.05$) was recorded in CFC samples compared to others.

3.6. Psychrotrophic bacteria count in bulk tank milk samples

Psychrotrophic bacteria count (PBC) was examined in 4 bulk tank milk samples. Three samples were originated from Farm III and one sample from IV. According to this study, PBCs were 4.3 ± 1.0 and 3.6 CFU/mL in BTM samples of Farm III and IV respectively. A non-significant strong positive correlation ($r = 0.693$; $P = 0.307$) was observed between total plate count and psychrotrophic bacterial counts evaluated in four BTM samples.

3.7. Lactic acid bacteria in milk and cheese samples

Lactic acid bacteria count (LABC) was tested in only nine individual animal raw milk samples originated from Farm I in 2020 with a mean value of 5.0 ± 0.6 lg CFU/mL. Milk samples from Farm II were not tested. The result of LABC in the IRM samples from Farm III (n = 10) and IV (n = 10) was the same (3.3 lg CFU/mL). LABC in BTM obtained from Farm III (n = 3) and IV (n = 1) was 6.7 ± 0.4 and 4.2 lg CFU/mL respectively. Lactic acid bacteria count was enumerated for six cheese samples out of 15. The mean of LABC was 7.5 ± 0.1 and 6.5 ± 0.3 lg CFU/g in GFSC and GFC, respectively. There was significant difference ($P < 0.05$) between two types of cheese samples. LABC value in GFC was significantly lower ($P < 0.05$) compared to GFSC.

3.8. Correlation between total plate count and *Enterobacteriaceae* count

In our study, weak to moderate positive correlations were observed between the bacterial count pairs evaluated. TPC^{IRM} had a significantly ($P < 0.01$) weak-positive correlation with EBC^{IRM} ($r = 0.295$). The same is true for TPC^{US} and EBC^{US} ($r = 0.323$, $P < 0.01$). As it was expected, EBC^{US} revealed a significantly ($P < 0.01$) moderate-positive correlation with EBC^{IRM} ($r = 0.565$). There was also a weak positive correlation between TPC^{US} and EBC^{IRM} ($r = 0.271$, $P < 0.05$).

Table 8: Pearson correlation coefficients (r) between major bacterial counts of udder surface samples (n = 77) and individual raw milk samples (n = 77)

	TPC^{IRM}	TPC^{US}	EBC^{IRM}	EBC^{US}
TPC^{IRM}		0.185	0.295^{**}	0.025
TPC^{US}			0.271^{*}	0.323^{**}
EBC^{IRM}				0.565^{**}
EBC^{US}				

*P < 0.05; **P < 0.01. TPC: total plate count; EBC: *Enterobacteriaceae* count; ^{IRM}: individual raw milk; ^{US}: udder surface.

3.9. Microbiota and taxonomic profile of five ovine milk samples

Four bacterial phyla (*Actinobacteria*, *Bacteroidetes*, *Firmicutes,* and *Proteobacteria)* were present in all milk samples analysed for microbiota taxonomic profile. The dominating microbiota at the phylum level was *Proteobacteria* in the three milk samples with a relative abundance of 98.8, 52.5, and 45.0% in IRM2, FBTM, and CTM1 respectively. *Firmicutes* and ‘’others’’ (all taxonomic units with less than 0.0% of reads at phylum level are collapsed in the category) were abundant in the CTM2 (82.4%) IRM1 and (47.5%) sample, respectively. *Bacteroidetes* was the second abundant microbiota in CTM1 (37.1%) and FBTM (29.4%) at the phylum level. However, the milk sample of IRM1 was dominated by *Proteobacteria* phylum (41.1%) next to others. *Firmicutes* was another phylum, the third dominant microbiota in FBTM (17.4%) and CTM1 (15.9%) samples whereas *Actinobacteria* (14.8%) in the IRM1 sample.

According to the analyses, the IRM2 and FBTM samples were dominated by the genera *Pseudomonas*, accounting for 40.8 and 35.5%, followed by *Chryseobacterium* (32.2%) and *Acinetobacter* (31.5%) respectively (Figure 17). *Chryseobacterium* (43.0%), *Ralstonia* (12.8%), and *Ottowia* (9.6%) genera dominated the sample of milk from the cooling tank (CTM1). Next to ‘’others’’ groups of bacterial genera, *Lonsdalea* (22.5%) was the second abundant genus in IRM1 sample followed by *Acinetobacter* (19.0%) and *Microbacterium* (17.4%).

3.10. Characteristics of *S. aureus* and coagulase-negative staphylococci strains

All *S. aureus* (n = 29) had positive reactions for coagulase and latex agglutination test. Out of 29 *S. aureus* isolates, 72.4% (n = 21) and 27.6% (n = 8) of *S. aureus* strains were confirmed by MALDI-TOF and API Staph for identification, respectively (Table 9). All *S. aureus* isolates tested for hemolysis were able to produce either both α and β or one among the two-hemolysis types. In our study, all *S. aureus* strains were sensitive to five of eight antibiotics tested. Out of 29 *S. aureus* isolates, 6 (20.7%) were resistant to tetracycline, whereas three and one *S. aureus* strains were intermediately resistant to erythromycin and clindamycin respectively (Table 9). Staphylococcal enterotoxin genes were detected in 25 *S. aureus* isolates in which *sec* was the most prevalent, being detected in 17 *S. aureus* strains.

Out of 16 CNS strains considered for characterization, 50% (n = 8), 25% (n = 4), and 25% (n = 4), were originated from strains identified by 16S rRNA gene sequencing, API Staph and MALDI-TOF MS Biotyper, and, respectively (Table 10). Out of 16 CNS isolates tested for hemolysis, eight were able to produce weak α or β or γ hemolysis types. In the present study, out of 16 CNS strains tested for antibiotic resistance against eight antibiotics, in five (31.2%) of CNS strains, at least one antibiotic resistance was detected (Table 10). CNS isolates were resistant to penicillin G (18.7%), tetracycline (12.5%), sulfamethoxazole (12.5%) and erythromycin (6.2%) (Table 10). Enterotoxin gene (*seg* and *sei*) was diagnosed in only one CNS strain (*S. auricularis*).

Table 9: Characteristics of *Staphylococcus aureus* isolates from ovine-associated samples

Origin	Type of sample	Strains ID	Tellurite reduction	Lecithinase activity	Hemolysis	Antibiotic test	Catalase test	Oxidase test	Enterotoxins
FI	US	SAA22	black	-ve	α-β	I (E)	+ve	-ve	SEC
FI	US	SAA23	black	-ve	α-β	S	+ve	-ve	SEC
FI	IRM	SAA1	black	-ve	α-β	S	+ve	-ve	SEC
FI	IRM	SAA2	grey	+ve	α-β	S	+ve	-ve	SEC
FI	IRM	SAA17	black	+ve	α-β	S	+ve	-ve	SEB, SEG, SEI
FI	IRM	SAA18	black	-ve	α-β	S	+ve	-ve	SEB
FI	IRM	SAA19	black	-ve	α-β	S	+ve	-ve	SEB
FI	IRM	SAA20	black	-ve	α-β	S	+ve	-ve	SEB
FI	IRM	SAA21	dark-black	-ve	α-β	S	+ve	-ve	SEB
FI	IRM	SAA24	dark-black	-ve	α-β	S	+ve	-ve	SEC
FIII	IRM	SAA7	black	-ve	α-β	I (E)	+ve	-ve	SEC
FIII	IRM	SAA8	black	-ve	α-β	S	+ve	-ve	SEC
FIII	IRM	SAA9	grey	-ve	α-β	S	+ve	-ve	SEC
FIII	IRM	SAA10	black	-ve	α-β	S	+ve	-ve	SEC
FIII	IRM	SAA26	black	-ve	weak β	R (TE)	+ve	-ve	SEC
FIII	IRM	SAA27	black	-ve	α-β	S	+ve	-ve	SEC
FIII	BTM	SAA3	black	-ve	α	I (DA), R (TE)	+ve	-ve	SEC

Origin	Type of sample	Strains ID	Tellurite reduction	Lecithinase activity	Hemolysis	Antibiotic test	Catalase test	Oxidase test	Enterotoxins
FIII	BTM	SAA4	black	-ve	α-β	R (TE)	+ve	-ve	SEC
FIII	BTM	SAA5	black	+ve	α-β	R (TE)	+ve	-ve	SEC
FIII	BTM	SAA6	black	+ve	α-β	R (TE)	+ve	-ve	SEB, SEI
FIII	BTM	SAA13	black	-ve	α	S	+ve	-ve	-
FIII	BTM	SAA14	black	-ve	α	S	+ve	-ve	-
FIII	BTM	SAA25	black	-ve	weak β	R (TE)	+ve	-ve	SEC
FIV	IRM	SAA11	black	-ve	α-β	S	+ve	-ve	SEC
FIV	IRM	SAA12	black	-ve	α-β	S	+ve	-ve	SEC
OMÉK	Cheese	SAA15	dark-black	+ve	β	I (E)	+ve	-ve	-
OMÉK	Cheese	SAA16	black	-ve	β	S	+ve	-ve	SEG, SEI
OMÉK	Cheese	SAA28	black	-ve	β	S	+ve	-ve	-
OMÉK	Cheese	SAA29	black	-ve	β	S	+ve	-ve	SEG, SEI

FI: Farm I; FIII: Farm III; FIV: Farm IV; US: udder surface; IRM: individual raw milk; BTM: bulk tank milk; OMÉK: 79th National Agriculture and Food Exhibition and Fair held in Budapest, Hungary, in September 2019; S: susceptible; I: intermediate; R: resistant; DA: clindamycin; E: erythromycin; TE: tetracycline.

Table 10: Characteristics of coagulase-negative staphylococcal isolates from ovine-associated samples

Farm	Origin of isolates	Strains ID	Organisms	Tellurite reduction	Lecithinase activity	Hemolysis	Antibiotic test	Catalase test	Oxidase test	Enterotoxins
FI	US	CNS4	*S. equorum*	black-yellow	-ve	weak α	I (DA, E)	+ve	-ve	-
FI	US	CNS8	*S. equorum*	black	-ve	-	S	+ve	-ve	-
FI	IRM	CNS1	*S. auricularis*	black	-ve	weak γ	R (SXT)	+ve	-ve	SEG, SEI
FI	IRM	CNS2	*S. equorum*	black	-ve	weak α	I (E)	+ve	-ve	-
FI	IRM	CNS3	*S. auricularis*	grey-whitish	-ve	-	R (SXT)	+ve	-ve	-
FI	IRM	CNS5	*S. simulans*	white	-ve	-	S	+ve	-ve	-
FI	IRM	CNS6	*S. simulans*	white	-ve	-	S	+ve	-ve	-
FI	IRM	CNS7	*S. simulans*	grey	-ve	-	S	+ve	-ve	-
FII	IRM	CNS15	*S. xylosus*	black	-ve	weak α	S	+ve	-ve	-
FII	IRM	CNS16	*S. simulans*	black	-ve	weak α	S	+ve	-ve	-
FIII	IRM	CNS10	*S. simulans*	grey	-ve	-	I (SXT)	+ve	-ve	-
FIII	IRM	CNS13	*S. caprae*	black	-ve	very weak β	R (P, TE)	+ve	-ve	-
FIII	IRM	CNS14	*S. caprae*	black	-ve	very weak β	R (P, E)	+ve	-ve	-
FIII	BTM	CNS9	*S. haemolyticus*	black	-ve	-	I (DA), R (P, TE)	+ve	-ve	-
FIV	US	CNS12	*S. auricularis*	black	-ve	weak α	I (SXT)	+ve	-ve	-
FIV	IRM	CNS11	*S. simulans*	grey	-ve	-	I (SXT)	+ve	-ve	-

FI: Farm I; FII: Farm II; FIII: Farm III; FIV: Farm IV; US: udder surface; IRM: individual raw milk; BTM: bulk tank milk; S: susceptible; I: intermediate; R: resistant; P: penicillin G; DA: clindamycin; E: erythromycin; TE: tetracycline; SXT: sulfamethoxazole/trimethoprim.

3.11. Phenotypical characteristics of lactic acid bacteria strains

Eleven lactic acid bacteria strains identified by MALD-TOF from ovine milk and cheese samples were tested for catalase test, oxidase test and antibiotic resistance to 10 antibiotics by disc diffusion method on MRS agar. In this study, seven LAB isolates were resistant to vancomycin which is not considered a health risk, according to WHO. As a result, vancomycin (VA) resistance was not included in the overall resistance of lactic acid bacteria, despite the fact that seven LAB isolates were resistant. Eight (72.7%) of 11 lactic acid bacteria isolates were identified to be resistant to cefoxitin. Only one strain (LAB5) was resistant against tetracycline (Table 11).

Table 11: Characteristics of lactic acid bacteria strains

Origin	Sample type	Analyte ID	Organism	Catalase test	Oxidase test	Antibiotic resistance profile*
Farm III	IRM	LAB5	*Lactobacillus plantarum*	-ve	-ve	R (FOX, TE, VA), I (P)
Farm III	IRM	LAB19	Presumably *Lactobacillus paracasei*	-ve	-ve	R (FOX, VA)
Farm III	BTM	LAB4	Presumably *Lactobacillus paracasei*	-ve	-ve	R (FOX, VA)
Farm III	BTM	LAB16	Presumably *Leuconostoc lactis*	-ve	-ve	Susceptible
OMÉK	Cheese	LAB28	Presumably *Lactobacillus plantarum*	-ve	-ve	R (FOX, VA), I (P, TE)
OMÉK	Cheese	LAB29	*Enterococcus faecalis*	-ve	-ve	R (FOX), I (TE)
OMÉK	Cheese	LAB30	Presumably *Lactobacillus plantarum*	-ve	-ve	R (FOX, VA), I (P, S)
OMÉK	Cheese	LAB31	Presumably *Lactobacillus plantarum*	-ve	-ve	R (FOX, VA), I (P)
OMÉK	Cheese	LAB32	Presumably *Lactobacillus plantarum*	-ve	-ve	R (FOX, VA), I (P)
OMÉK	Cheese	LAB33	*Lactobacillus plantarum*	-ve	-ve	I (FOX, VA)
OMÉK	Cheese	LAB35	*Lactobacillus plantarum*	-ve	-ve	Susceptible

*Resistance to vancomycin in lactic acid bacteria is intrinsic, which is acceptable/not considered a health risk. Vancomycin resistant LAB strains were tot included in overall resistancy percentage. BTM: bulk tank milk; IRM: individual raw milk; FOX: cefoxitin; I: intermediate; P: penicillin G; R: resistance; S: streptomycin; TE: tetracycline; VA: vancomycin; OMÉK: 79th National Agriculture and Food Exhibition and Fair held in Budapest, Hungary, in September 2019.

4. CONCLUSIONS AND RECOMMENDATIONS

In conclusion, the presence of a positive California Mastitis Test score in few udder halves milk samples might indicates the good health condition of flocks. However, disinfecting the teats of the animal before and after milking and cleaning the udder surface can prevent the occurrence of subclinical mastitis on the farms, where positive results were detected. Relatively low bacterial counts in udder surface and individual raw milk samples have reflected good housing and health conditions of sheep flocks kept on the four farms studied. However, the presence of total plate count in bulk tank milk of two farms at levels exceeding regulatory limit, which is 6.2 lg CFU/mL for milk that will undergo pasteurization, is indicative of poor hygienic standards during milking and on-farm milk handling. Therefore, measures should be taken to improve the sanitary conditions of ovine milk production on the studied farms. Besides, the present delivery practice of bulk tank milk from the farm to processing units should be revised. The length of storage of milk on the farm should be minimized by more frequent delivery of fresh milk to the dairy processing plant.

In the case of some ovine cheese samples, the high counts of *Enterobacteriaceae* and *Escherichia coli* were a sign of the existence of safety risk. The higher value of *Staphylococcus aureus* in chives-flavoured cheese samples may also indicate the unhygienic nature of ovine-milk cheese. As a result, awareness should be raised on the importance of some pre-requisites and process control points. For example, the utilization of high-quality raw materials, production in fashionable enterprises, and healthful conditions are going to be effective in minimizing contamination of bacterium.

In this study, a significant correlation between udder surface and individual raw milk samples for *Enterobacteriaceae* count indicates that unhygienic farm environments, such as dirty bedding, are prejudicing factors for the occurrence of unwanted microorganisms in unpasteurized ovine milk. Four bacterial phyla (*Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*) were present in five milk sample types analysed for microbiota taxonomic profile. Due to the small sample size, microbiota richness and diversity indexes based on three milk sample types were not determined. This suggesting that sufficient sample size from different milk and environmental sample types should be considered in future for ovine-associated microbiota community studies.

Furthermore, the resistance against tetracycline was observed in six *S. aureus*, and penicillin G, tetracycline, trimethoprim/sulfamethoxazole, and erythromycin resistance was detected in at least one of five resistant coagulase-negative staphylococci strains. Eight lactic acid bacteria strains were resistant to at least one of the two antibiotics (cefoxitin and tetracycline). Also, SE genes were detected in 25 *Staphylococcus aureus* and one coagulase-negative *Staphylococcus* strains in which SEC was the most prevalent, being detected in 17 *Staphylococcus aureus* strains. Although the farm manager was not willing to tell the type of antibiotics used in the above-mentioned farm, the animals could be treated with antibiotics in which resistant strains were found. The presence of antibiotic resistance and SEs genes in the raw milk and cheese originated strains affects consumer’s health. The interventions are needed to prevent the occurrence of pathogenic staphylococci in dairy ovine farms to ultimately prevent the milk supply from contamination with these bacteria. Moreover, careful use of antibiotics by veterinarians, avoiding raw milk consumption, and implementing a surveillance system to monitor staphylococcal enterotoxins in the ovine milk chain are of utmost crucial to minimize public health risk.

5. NEW SCIENTIFIC RESULTS

1. Total plate count was 2.9 ± 1.0 lg CFU/mL, *Enterobacteriaceae* count: 1.5 ± 0.5 lg CFU/mL and *Staphylococcus aureus* count: 2.6 ± 0.7 lg CFU/mL in overall (n = 108) California Mastitis Test negative udder halves milk samples. Individual raw milk samples with weak positive (n = 11) and positive (n = 5) CMT scores had TPC mean value of 3.6 ± 1.1 and 3.6 ± 0.8 lg CFU/mL, *Enterobacteriaceae* count: 1.8 ± 0.6 and 3.0 ± 0.9 lg CFU/mL, *Staphylococcus aureus* count: 2.8 ± 0.3 and 3.4 lg CFU/mL, respectively.

2. The overall mean value of total plate count of udder surface samples obtained from four sheep farms was 2.5 ± 0.8 lg CFU/cm^2 (ranged from 1.0 to 5.1 lg CFU/cm^2). *Enterobacteriaceae* count of udder surface samples was 1.2 ± 0.6 lg CFU/cm^2 (ranged from 0.1 to 2.6 lg CFU/cm^2). *S. aureus* was detected in only 2.6% (n = 2/77) of udder surface samples.

3. Overall average value of total plate count of individual raw milk samples originated from four sheep farms was 3.1 ± 1.1 lg CFU/mL (ranged from 1.0 to 5.5 lg CFU/mL). *Enterobacteriaceae* count of individual raw milk samples was 1.9 ± 0.8 lg CFU/mL (ranged from 0.0 to 3.9 lg CFU/mL). *S. aureus* was detected in six (7.0%) individual raw milk samples with overall mean value of 2.9 ± 0.6 lg CFU/mL.

4. Six (20.7%) *S. aureus* isolates of Farm III (five isolates of bulk tank milk and one of individual raw milk) were resistant to tetracycline. Staphylococcal enterotoxin genes were detected in 25 *S. aureus* isolates in which *sec* was the most prevalent, being detected in 17 *S. aureus* strains. Higher occurrence of enterotoxins was observed in strains originated from Farm I (n = 11/17) and III (n = 11/18), followed by Farm IV (n = 2/4) and Farm 2 (n = 0/2). Enterotoxins detected more frequently in IRM (n = 17) followed by BTM (n = 5) and cheese (n = 2) and udder surface (n = 2) samples.

5. Six coagulase-negative *Staphylococcus* (*S. auricularis, S. caprae, S. equorum, S. haemolyticus, S. simulans* and *S. xylosus*) were identified from either from udder surface and/or from raw milk samples. Penicillin G, tetracycline, sulfamethoxazole, and erythromycin antibiotics resistance was detected in at least one of five resistant coagulase-negative staphylococci strains. Among the five resistant coagulase-negative

staphylococci isolates, three were originated from Farm III (2 strains of individual raw milk and 1 of bulk tank milk) and two were from Farm I individual raw milk. Enterotoxin gene (*seg* and *sei*) was diagnosed in only one coagulase-negative *Staphylococcus* strain (*S. auricularis*) of Farm I individual raw milk.

6. Three different lactic acid bacteria genera were identified namely *Lactobacillus*, *Leuconostoc* and *Enterococcus*. Eight (72.7%) lactic acid bacteria strains, five from cheese samples and three from Farm III milk samples, were resistant to cefoxitin. Out of the eight cefoxitin resistant isolates, one was also resistant to tetracycline.

6. RESULTS APPLICABLE IN PRACTICE

1. Total plate count in bulk tank milk of two farms (Farm II and III) where it exceeded regulatory limit, which is 6.2 lg CFU/mL for milk that will undergo pasteurization can be improved by improving sanitary conditions and delivering fresh milk more frequently to dairy processing plants. In the case of some ovine cheese samples, the high counts of *Staphylococcus aureus*, *Enterobacteriaceae* and *Escherichia coli* might be minimized through the utilization of high-quality raw materials (such as pasteurized milk), production in fashionable enterprises, and healthful conditions.

2. The identification and characterization of staphylococcal and lactic acid bacteria strains is crucial in dairy microbiology in order to fully understand the properties of the bacterial strains and to prevent contamination of ovine milk and cheese effectively.

3. Antibiotic-resistant staphylococcal strains were discovered in some of milk samples from Farms I and III. Moreover, resistant (except intrinsic resistance against vancomycin) lactic acid bacteria strains were found in some milk samples of Farm III and cheese samples. Disease prevention, avoiding misuse and overuse of antibiotic in accordance with WHO guideline (2017) on use of antibiotics in food producing-animals can all help to reduce the incidence.

4. Staphylococcal enterotoxin gene producing strains were found in some of udder surface, milk and cheese samples. According to COMMISSION REGULATION (EC) 2073/2005 on microbiological criteria for foodstuffs, dairy and dairy products sample should be free of enterotoxins. Even though, there is no clear staphylococcal enterotoxin concentration limit value, studies suggested that staphylococcal enterotoxin production is correlated with bacterial growth, i.e., the more bacterial counts, the more toxin production. Therefore, hygienic measures such as storing pasteurized milk and the dairy product below 6-7 ^{o}C, pasteurization of raw milk, and personal hygiene during milking can be taken to reduce *Staphylococcus* contamination.

7. REFERENCES

1. BANIA, J.–DABROWSKA, A.–BYSTRON, J.–KORZEKWA, K.–CHRZANOWSKA, J.–MOLENDA, J. (2006): Distribution of newly described enterotoxin-like genes in *Staphylococcus aureus* from food. International Journal of Food Microbiology. 108, 36–41.
2. CHIANG, Y.–LIAO, W.–FAN, C.–PAI, W.–CHIOU, C.–TSEN, H. (2008): PCR detection of staphylococcal enterotoxins (SEs) N, O, P, Q, R, U, and survey of SE types in *Staphylococcus aureus* isolates from food-poisoning cases in Taiwan. International Journal of Food Microbiology. 121, 66–73.
3. CLINICAL AND LABORATORY STANDARDS INSTITUTE (CLSI). (2017): Performance standards for antimicrobial susceptibility testing; 7th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute, USA.
4. COMMISSION OF THE EUROPEAN COMMUNITIES. COMMISSION REGULATION (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Official Journal of the European Union. L338, 1–26.
5. FOOD and AGRICULTURE ORGANIZATION of the UNITED NATIONS (FAO). (2019): FAOSTAT database: Livestock primary. Available online: http://www.fao.org/faostat/en/#data/QL (retrieved on 20 January 2021).
6. HUNGARIAN CENTRAL STATISTICAL OFFICE (KSH). (2019): Sheep stock. Available online: http://www.ksh.hu/docs/eng/xstadat/xstadat_infra/e_oma006.html?lang=en (retrieved on 20 January 2021.
7. HUNGARIAN SHEEP and GOAT BREEDERS' ASSOCIATION (HSGBA). Magyar Juh-és Kecsketenyésztő Szövetség. Available online: https://mjksz.hu/fajta/keletfriz (retrieved on 16 September 2020.
8. GONZALO, C. (2017): Milk hygiene in small ruminants: A review. Spanish Journal of Agricultural Research. 15, e05R02.
9. INTERNATIONAL ORGANIZATION for STANDARDIZATION (ISO). ISO 707:2008 (International Dairy Federation 50: 2008). Milk and milk products-guidance on sampling. Switzerland, Geneva.
10. INTERNATIONAL ORGANIZATION for STANDARDIZATION (ISO) 4833–1. Microbiology of the food chain-Horizontal method for enumeration microorganisms–Part 1: Colony count at 30 °C by the pour plate technique. 2014. (ISO 4833–1:2013).

11. ISO 21528–2. Microbiology of the food chain-Horizontal method for the detection and enumeration of *Enterobacteriaceae*–Part 2: Colony-count technique. 2017. (ISO 21528–2:2017).
12. ISO 6888–1. Microbiology of food and feed-Horizontal method is to determine the number of coagulase-positive staphylococci–Part 1: Technique using Baird-Parker agar medium. 2008. Amendment 1: Inclusion of precision data (ISO 6888–1:1999/Amd.1:2008).
13. ISO 16649–2. Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli*–Part 2: Colony-count technique at 37°C. (ISO 16649–2:2001).
14. ISO 15214. Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of mesophilic lactic acid bacteria. Colony-count technique at 30 °C. (ISO 15214:2005).
15. ISO 17410–2. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of psychrotrophic microorganisms. (ISO 17410–2:2005).
16. KAČÁNIOVÁ, M.–KUNOVÁ, S.–SABO, J.–IVANIŠOVÁ, E.–ŽIAROVSKÁ, J.–FELŠÖCIOVÁ, S.–FATRCOVÁ–ŠRAMKOVÁ, K.–TERENTJEVA, M. (2020): Isolation and identification of lactic acid bacteria in wine production by MALDI-TOF MS Biotyper. The Scientific Journal for Horticulture, Landscape Engineering and Architecture. 1 21–24.
17. KUKOVICS, S.–NÉMETH, T.–ÁBRAHÁM, M.–ORBÁN, G. (2009): A juh- és a kecsketej min″osége (Quality of ovine and caprine milk). In A Tej Szerepe a Humán Táplálkozásban (Role of Milk in Human Nutrition); Kukovics, S., Ed. Melánia Kiadó: Budapest, Hungary, pp401–416.
18. LANE, D.J. (1991): 16S/23S rRNA Sequencing. In: Stackebrandt, E. and Goodfellow, M., Eds., Nucleic acid techniques in bacterial systematic, John Wiley and Sons, New York. 115–175.
19. MEHROTRA, M.–WANG, G.–JOHNSON, W.M. (2000): Multiplex PCR for detection of genes for *Staphylococcus aureus* enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. Journal of Clinical Microbiology. 38, 1032–1035.
20. PARK, Y.W.–ALBENZIO, M.–SEVI, A.–HAENLEIN, G.F. (2013): Milk quality standards and controls. In: Park, Y.W.–Haenlein, G. (Eds.), Milk and dairy products in human nutrition: production. Composition and health. Wiley, Oxford, pp261–287.

21. PEXARA, A.–SOLOMAKOS, N.–SERGELIDIS, D.–ANGELIDIS, A.S–GOVARIS, A. (2016): Occurrence and antibiotic resistance of enterotoxigenic *Staphylococcus aureus* in raw ovine and caprine milk in Greece. Dairy Science and Technology. 96, 345–357.
22. PIRISI, A.–LAURET, A.–DUBEUF, J.P. (2007): Basic and incentive payments for goat and sheep milk in relation to quality. Small Ruminant Research. 68, 167–178.
23. SHARMA, N.K.–REES, C.E.–DODD, C.E. (2000): Development of a single-reaction multiplex PCR toxin typing assay for *Staphylococcus aureus* strains. Applied and Environmental Microbiology. 66, 1347–1353.
24. STATISTICAL PACKAGE FOR THE SOCIAL SCIENCES (SPSS). (2019): IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.
25. WORLD HEALTH ORGANIZATION (2017): WHO guidelines on use of medically important antimicrobials in food-producing animals. Geneva: World Health Organization; 2017. Licence: CC BY-NC-SA 3.0 IGO.

8. PUBLICATIONS ON THE TOPIC OF THE DISSERTATION

UNIVERSITY of DEBRECEN

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

Registry number: DEENK/355/2021.PL
Subject: PhD Publication List

Candidate: Andualem Tonamo
Doctoral School: Doctoral School of Animal Husbandry
MTMT ID: 10061765

List of publications related to the dissertation

Foreign language scientific articles in Hungarian journals (3)

1. **Tonamo, A.**, Komlósi, I., Szabóné Petróczki, F. M., Peles, F.: Coagulase-negative staphylococci in ewe udder surface and raw milk samples.
   Agrártud. Közl. 1, 221-225, 2021. ISSN: 1587-1282.
   DOI: http://dx.doi.org/10.34101/actaagrar/1/8503

2. **Tonamo, A.**, Komlósi, I., Varga, L., Kačániová, M., Peles, F.: Identification of ovine-associated staphylococci by MALDI-TOF mass spectrometry.
   Acta Aliment. 50 (2), 210-218, 2021. ISSN: 0139-3006.
   DOI: http://dx.doi.org/10.1556/066.2020.00246
   IF: 0.458 (2019)

3. **Tonamo, A.**, Komlósi, I., Peles, F.: Bacteria in the milk of sheep with or without mastitis- mini Review.
   Agrártud. Közl. 2019 (1), 47-52, 2019. ISSN: 1587-1282.
   DOI: http://dx.doi.org/10.34101/actaagrar/1/2369

Foreign language scientific articles in international journals (1)

4. **Tonamo, A.**, Komlósi, I., Varga, L., Czeglédi, L., Peles, F.: Bacteriological Quality of Raw Ovine Milk from Different Sheep Farms.
   Animals (Basel). 10 (7), 1-9, 2020. ISSN: 2076-2615.
   DOI: http://dx.doi.org/10.3390/ani10071163
   IF: 2.323 (2019)

Foreign language conference proceedings (1)

5. **Tonamo, A.**, Komlósi, I., Szabóné Petróczki, F. M., Orosz, É., Aamir, M., Peles, F.: Microbiological quality of raw milk and udder surface samples from Dorper, Merino and Cigaja sheep breeds.
   In: Scientific researches in food production : 3rd meeting of young researchers from V4 countries /d. Béla Kovács, Nikolett Czipa, Ferenc Peles, Éva Bacskainé Bódi, Andrea Kántor, Flóra Mária Szabóné Petróczki, Loránd Alexa, University of Debrecen, Debrecen, 55-58, 2018. ISBN: 9789634900320

UNIVERSITY of DEBRECEN

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

Foreign language abstracts (3)

6. **Tonamo, A.**, Komlósi, I., Peles, F.: Microbiological properties of raw ewe milk and udder surface samples in a Hungarian dairy sheep farm.
   Acta Microbiol. Immunol. Hung. 66 (Suppl.), 118, 2019. ISSN: 1217-8950.

7. **Tonamo, A.**, Komlósi, I., Oláh, J., Peles, F.: Microbiological status of udder surface and raw ewe milk samples originated from different sheep breeds.
   In: Innovation challenges in the 21st century : LXI. Georgikon Napok International Scientific Conference : abstract volume. Eds.: Gábor Pintér, Szilvia Csányi, Henrik Zsiborács, University of Pannonia Georgikon Faculty, Keszthely, 111, 2019. ISBN: 9789633961292

8. **Tonamo, A.**, Komlósi, I., Peles, F.: The hygienical status of milk and environmental samples derived from different sheep breeds.
   In: Scientific researches in food production : Proceeding of abstracts, Debreceni Egyetem, Debrecen, 28, 2018.

UNIVERSITY of DEBRECEN

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

List of other publications

Foreign language scientific articles in Hungarian journals (2)

9. Zegeye, Z. B., Vass, N., **Tonamo, A.**: Application of laparoscopic artificial insemination in conventional Lacaune sheep farm using frozen-thawed semen.
Agrártud. Közl. 2020 (2), 133-138, 2020. ISSN: 1587-1282.
DOI: http://dx.doi.org/10.34101/ACTAAGRAR/2/7113

10. Szabóné Petróczki, F. M., **Tonamo, A.**, Béri, B., Peles, F.: The effect of breed and stage of lactation on the microbiological status of raw milk.
Agrártud. Közl. 1, 37-45, 2019. ISSN: 1587-1282.
DOI: http://dx.doi.org/10.34101/actaagrar/1/2367

Foreign language scientific articles in international journals (1)

11. **Tonamo, A.**, Berhan, T., Gebeyehu, G., Ermias, B.: Characterization of cattle husbandry practices in Essera Woreda, Dawuro Zone, Southern Ethiopia.
Afr. J. Agric. Res. 10 (34), 3421-3435, 2015. EISSN: 1991-637X.
DOI: http://dx.doi.org/10.5897/AJAR2015.9939

Total IF of journals (all publications): 2.781
Total IF of journals (publications related to the dissertation): 2.781

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

07 June, 2021