

## THE BACTERIOLOGICAL QUALITY OF GOAT AND OVINE MILK

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### ABSTRACT

This study concentrates on information concerning the microbiological hazards that can be present in raw milk from animal species other than cows. A total of 54 (23 of ovine and 31 of goat) bulk tank milk samples from 10 farms in the Czech Republic were collected in years 2013 – 2014. The sampling was done at regular time intervals during the whole year, with five to eight samples collected from each of the 10 dairy farms involved in the study. All milk samples were collected into sterile sampling bottles and transported in a cooler sampling case to the laboratory for immediate examination. Farms were randomly selected to cover the whole area of the Czech Republic. The prevalence and characteristic of *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., *Campylobacter* spp. and *Listeria monocytogenes* was studied. Raw cow's milk can be contaminated by *E. coli* intramammarily during clinical or subclinical mastitis and either directly through animal feces or indirectly during milk collection through farm employees or the milking equipment. *E. coli* was detected in 90.3% of the goat milk and 95.7% of the ovine milk samples. The genes encoding Shiga toxins 1 and 2– (*stx*<sub>1</sub>, *stx*<sub>2</sub>) were not detected and no STEC was identified. The *Eae* was detected in 3 (4.6%) isolates. *S. aureus* was detected in 9 (29.0%) samples of goat milk and 8 (34.8%) samples of ovine milk. A total 12 (57.1%) enterotoxin positive *S. aureus* were obtained; 6 (28.6%) were positive for the production of *sec* encoding enterotoxin SEC; in 4 (19.0%) isolates the gene *seh* was detected; 2 (9.5%) isolates were proven positive for *seg* (4.8%) and combination *seg* and *sei* (4.8%). The presence of MRSA was not detected in the tested samples in our study. *L. monocytogenes* was detected in 1 (3.2%) samples of goat milk and 1 (4.3%) samples of ovine milk. The serotype (1/2a, 1/2b) was detected in our study. *Campylobacter* spp. and *Salmonella* spp. were not isolated from any of the samples. These results form the basis for determining the microbiological quality standards for goat and ovine milk.

**Keywords:** bacteria; *Escherichia coli*; *Staphylococcus aureus*; *Salmonella* spp.; *Campylobacter* spp.; *Listeria monocytogenes*

### INTRODUCTION

This study concentrates on information concerning the microbiological hazards that can be present in raw milk from animal species other than cows. Milk and dairy products are basic components of human diet. Consumption of raw milk represents a risk for the consumers, due to the possible presence of human pathogenic microorganisms in raw milk (Claeys et al., 2013). Public health problems associated with consumption of unpasteurized cow's milk and raw-milk products have been well documented (Cody et al., 1999; Kalman et al., 2000; De Buyser et al., 2001; Harrington et al., 2002). Goat and ovine production constitutes an important part of the national economy in many countries. One of the most decisive factors in the growth in the consumption of goat and ovine milk is their perceived beneficial effect on human health, which, moreover, is fully recognized by the scientific community. Goat milk has an acceptable, attractive odour and taste, and can be consumed as an alternative to cow milk because it is less allergenic. Ovine milk has a higher content of essential vitamins and minerals than cow's milk and could be used to cater to consumers' appetite for healthy and safer products (Park et al., 2007).

In general, the scientific quality of research on goat and ovine milk products is still insufficient but is continuously improving.

Milk quality can be evaluated according to hygienic, nutritional, technological and sensory parameters. One of the most important criteria that determine goat and sheep's milk quality is the control of pathogens.

The objectives of this study were: 1) to determine the microbiological status of goat and ovine milk in the Czech Republic, 2) to study the prevalence of food-borne pathogens, especially *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* spp., and *Campylobacter* spp. and 3) typing of selected pathogens (serotyping, detection of SEs, *mecA* genes etc.).

### MATERIAL AND METHODOLOGY

**Samples collection.** A total of 54 bulk tank milk samples from 10 farms in the Czech Republic were collected in years 2013 – 2014. The sampling was done at regular time intervals during the whole year, with five to eight samples collected from each of the 10 dairy farms involved in the study. Farms were randomly selected to cover the whole area of the Czech Republic. All milk samples were collected into sterile sampling bottles and transported in a cooler sampling case to the laboratory for immediate

examination. The milk samples were tested for the presence of: *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., *Campylobacter* spp. and *Listeria monocytogenes* each test was performed on 25 ml of raw milk by means of qualitative methods.

**Isolation and identification of *Escherichia coli* (*E. coli*).** Detection of *E. coli* was carried out according to ISO 16649-1 with some slight modifications. The detection was performed after enrichment of 25 ml of milk in 225 ml of buffered peptone water (BPW, Oxoid, UK) at 37 °C for 24 hours followed by aerobic cultivation on Tryptone Bile X-glucuronide medium (TBX, Oxoid, UK) (44 °C for 24 hours). From each positive sample, one to three suspected *E. coli* isolates were used for confirmation. Confirmation of suspected colonies from TBX agar consisted of the detection of oxidase (OXItest, Pliva-Lachema, CZ) and production of indole (COLItest, Pliva-Lachema, CZ).

**Isolation and identification of *Staphylococcus aureus* (*S. aureus*).** Detection of *S. aureus* was carried out according to ISO 6888-1 with slight modification as follows: 25 ml of milk was diluted with 225 ml of buffered peptone water (Oxoid, UK) and homogenized. After enrichment at 37 °C overnight samples were cultivated on Baird - Parker agar (B-P, Oxoid, UK) supplemented with egg yolk-tellurite emulsion. From each plate, both the typical and atypical colonies were examined by plasmacoagulase test (Staphylo LA Seiken, DENKA SEIKEN Co. Ltd., Japan) and confirmation of suspected *S. aureus* strains was carried out by polymerase chain reaction (PCR) based on the detection of the species specific fragment SA442 (Martineau et al., 1998).

**Isolation and identification of *Salmonella* spp.** The detection of *Salmonella* spp. was carried out according to ISO 6579. At first, non-selective enrichment was performed in buffered peptone water (Oxoid, UK). This was followed by selective enrichment in two types of media Rappaport-Vassiliadis Soya Peptone Broth (RVS) and Muller-Kauffmann Tetrathionate-Novobiocin Broth (MKTTn, Oxoid, UK) simultaneously. Then isolation on media RAMBACH (MERCK, D) and XLD (Oxoid, UK) was performed.

**Isolation and identification of *Campylobacter* spp.** The detection of thermotolerant *Campylobacter* spp. was carried out according to ISO 10272-1. After enrichment, which was done in Bolton medium with horse blood (Oxoid, UK) and after 48 hours of cultivation at 42 °C suspension was inoculated on *Campylobacter* Selective Blood Free Agar (CCDA, Oxoid, UK) with incubation at 42 °C for 48 hours at micro-aerophilic conditions.

**Isolation and identification of *Listeria monocytogenes* (*L. monocytogenes*).** Detection of *L. monocytogenes* was performed according to ISO 11290-1 with a modification in the primary multiplication step which was carried out in the buffered peptone water (Oxoid, UK) at 37 °C for 24 hours. Secondary multiplication was done in Fraser broth (Oxoid, UK) at 37 °C for 24 hours and followed by aerobic cultivation on ALOA agar medium (BIO-RAD, FR) at 37 °C for 24 hours.

**Typing of bacteria.** More attention has been concentrated to the occurrence of methicillin-resistant *S. aureus* strains (MRSA) and *E. coli* with detection of selected genes encoding virulence factors.

For the determination of MRSA in *S. aureus* isolates polymerase chain reaction (PCR) for the detection of the *mecA* gene, which is responsible for the resistance to methicillin (Poulsen et al., 2003) was used.

For the detection of the genes encoding staphylococcal enterotoxins A–J multiplex PCR method previously published by Lovseth et al., (2004) was used.

PCR was also used for the detection of selected genes encoding virulence factors (*eae*, *hly*, *stx1*, *stx2*) in *E. coli*. The detection of the virulence genes was performed using multiplex PCR according to Fagan et al., (1999).

Typical colonies for *Listeria monocytogenes* were confirmed and serotyped by slide agglutination method using the commercially available antisera (DenkaSeiken, Japan) and verified by multiplex PCR (Doumith et al., 2004).

## RESULTS AND DISCUSSION

This study was focused to map the occurrence of bacteriological risks in raw goat and ovine milk. In total, 54 (31 of goat milk and 23 of ovine milk) samples of bulk tank milk collected from 10 (5 of goat farm and 5 of ovine farm) different farms and investigated in 2013 – 2014 in the Czech Republic. The detailed results are shown in Table 1.

Differences were observed in the bacteriological quality of raw milk collected on the different dairy farms. While milk from some farms was bacteriologically safe in milk samples from other farms pathogenic microorganisms were repeatedly detected.

In our study, *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes* were detected.

**Prevalence of *E. coli* in raw milk.** Raw cow's milk can be contaminated by *E. coli* intramammarily during clinical and subclinical mastitis (Dahmen et al., 2013) and either directly through animal feces or indirectly during milk collection through farm employees or the milking equipment (Desmarchelier a Fegan, 2011). This study shows that the presence of *E. coli* in raw milk is very common. Altogether, 49 (90.7%) positive milk samples were detected. A total of 65 *E. coli* isolates were retained for further characterization. *E. coli* strains producing Shiga toxins (Stx) 1 and 2, encoded by *stx1* and *stx2* genes, respectively, are called Shiga toxin-producing *E. coli* (STEC) (Wani et al., 2009). These toxins have acytopathic effect on intestinal epithelial cells that plays a role in the development of bloody diarrhea. STEC have other, additional virulence factors, the most important of these is a protein called intimin (Robati a Gholami, 2013). Many STEC produce intimin, an adhesive protein encoded by the *eae* gene with several variants located on the pathogenicity island termed the locus of enterocyte effacement (Blanco et al., 2004). Of the adhesin coding genes, *eae* was the detected in 3 (4.6%) isolates. The genes encoding Shiga toxins 1 and 2– (*stx1*, *stx2*) were not detected and no STEC was identified.

**Table 1.** Results of microbiological quality of raw milk testing

Milk	Samples n	<i>Escherichia coli</i>		<i>Staphylococcus aureus</i>		<i>Listeria monocytogenes</i>	
		n	%	n	%	n	%
Goat milk	31	28	90.3	9	29.0	1	3.2
Ovine milk	23	22	95.7	8	34.8	1	4.3
Σ	54	50	92.6	17	31.5	2	3.7

**Prevalence of *S. aureus* in raw milk.** *Staphylococcus aureus* is one of the most important mammary gland pathogens responsible for mastitis that can cause enormous economic losses (Hata et al., 2008). When investigating the incidence of pathogenic microorganisms, we recorded the highest detection rate of *Staphylococcus aureus*. Altogether, 17 (31.5%) positive milk samples were detected. A total of 21 *S. aureus* isolates were retained for further characterization and detection enterotoxins encoding genes. Global problem of the 21st century becomes the occurrence of pathogenic microorganisms resistant to routinely used antibiotics. *S. aureus* has an impressive ability to adapt to environmental conditions and it can fast become resistant to almost all antibiotics (McCallum et al., 2010). Methicillin-resistant *S. aureus* (MRSA) were found primarily in humans, later they were detected also in animals (Lee et al., 2004). In recent years, the increase of staphylococci strains that show resistance to methicillin/oxacillin has become a serious clinical and epidemiological problem. Methicillin-resistant *S. aureus* in milk are less important as a food safety issue, since milk is almost always heat treated before consumption. However, these exceptions and raw milk consumption, which is widely practiced by farmers and their families (Oliver et al., 2009), could expose people to MRSA. Recent reports revealed that MRSA was also associated with cases of bovine and caprine mastitis (Aras et al., 2012; Vanderhaeghen et al., 2010). Occurrence of MRSA in goat milk has been observed in the Czech Republic, namely in 2008 (Štásková et al., 2009). The presence of MRSA was not detected in the tested samples in our study.

**Detection of enterotoxins in *S. aureus* isolates.** *Staphylococcus aureus* is an important human and animal pathogen known to produce a range of toxic substances that can cause various diseases. From the perspective of food microbiology, the most relevant characteristic of *S. aureus* is the production of heat-stable enterotoxins implicated in food-borne intoxications (Thomas et al., 2007). In terms of risk of foodborne diseases very important is the ability of approximately 50-75% of *S. aureus* strains to produce under the suitable conditions the extracellular thermostable enterotoxins (SEs) (Argudín et al., 2010).

From 54 milk samples examined, 17 (31.5%) positive

milk samples were detected. A total of 21 *S. aureus* were isolated and used for the detection of SEs. For the detection of the genes encoding enterotoxins A–J multiplex PCR was used (Lovseth et al., 2004).

A total 12 (57.1%) enterotoxin positive *S. aureus* were obtained. In our study, 6 (28.6%) of these isolates were positive for the production of classical enterotoxins SEA-SEE, which are the leading cause of foodborne diseases. In our study 6 of these isolates were positive for the production of *sec* encoding enterotoxin SEC.

It was also reported as the most frequent gene in *Staphylococcus aureus* from goat's milk from Scherrer et al., (2004) and Lyra et al., (2013), who observed the SEC in 42% and 55.6% of staphylococci. The presence of this gene was observed in 71% (Mork et al., 2010) and 86% (Silva et al., 2005) of *Staphylococcus aureus* strains isolated from healthy goats and goats with udder infections.

In our study, the 4 (19.0%) of these isolates were positive for the gene *seh* responsible for the production of SEH. Out of 21 strains investigated 2 (9.5%) were proven positive for *seg* (4.8%) and combination *seg* and *sei* (4.8%). No genes for SEs were identified in 9 (42.9%) isolates. Results are shown in Table 2.

Ikeda et al., (2005) described a mass outbreak of food poisoning caused by eating reconstituted milk contaminated by toxigenic *S. aureus* with co-productions of the SEA and SEH. The outbreak was also caused by SEH subsequently produced by *S. aureus* isolated from potato mash with raw milk (Jørgensen et al., 2005).

**Prevalence of *L. monocytogenes* in raw milk.** *Listeria monocytogenes* was detected in 2 (3.7%) samples. The prevalence of *L. monocytogenes* in bulk tank milk is reported to range from 1 to 12% (Oliver et al., 2005). Ninety-five percent of *L. monocytogenes* strains associated with human listeriosis and food samples belong to serotypes 1/2a, 1/2b, and 4b (Kathariou 2002). Serotype 1/2a has frequently been detected in different food matrices (Martins a Leal Germano, 2011). The same serotype (1/2a, 1/2b) was detected in our study.

**Prevalence of *Salmonella* spp. and *Campylobacter* spp. in raw milk.** The presence of *Salmonella* spp. and *Campylobacter* spp. was not detected in the tested samples.

**Table 2.** Prevalence of *S. aureus* with production staphylococcal enterotoxin genes.

Combination of toxins	Goat milk		Ovine milk	
	n	%	n	%
C	3	14.3	3	14.3
H	1	4.8	3	14.3

n- *S. aureus* with genes encoding SEs

## CONCLUSION

The results of this study confirm the presence of pathogenic bacteria in goat and ovine raw milk. The study shows the fact that the consumption of raw milk is not safe for the consumers, and that heat treatment of raw milk before the consumption has a positive meaning.

Our results confirm that unpasteurized milk may be contaminated with different types of microorganisms and can be an important source of foodborne illnesses. The most effective tool for the microbiological safety of milk is pasteurization or other heat treatment. Information on health hazards associated with contaminated raw milk should be extended to the public, so that consumption of untreated raw milk could be avoided.

## REFERENCES

- Aras, Z., Aydinb, I., Kav, K. 2012. Isolation of methicillin-resistant *Staphylococcus aureus* from caprine mastitis cases. *Small Ruminant Research*. vol. 102, no. 1, p. 68-73. <http://dx.doi.org/10.1016/j.smallrumres.2011.08.014>
- Argudín, M. A., Mendoza, M. C., Rodicio, M. R. 2010. Food Poisoning and *Staphylococcus aureus* enterotoxins. *Toxins*. vol. 2, no. 7, p. 1751-1773. <http://dx.doi.org/10.3390/toxins2071751> PMID:22069659
- Blanco, M., Blanco, J. E., Mora, A., Dahbi, G., Alonso, M. P., Gonzalez, E. A., Bernardez, M. I., Blanco, J. 2004. Serotypes, virulence genes and intimin types of Shiga toxin (Verotoxin)-producing *Escherichia coli* isolates from cattle in Spain: identification of a new intimin variant gene (eae-ζ). *J. Clin. Microbiol.*, vol. 42, no. 2, p. 645-651. <http://dx.doi.org/10.1128/JCM.42.2.645-651.2004> PMID:14766831
- Cody, S. H., Abbott, S. L., Marfin, A. A., Schulz, B., Wagner, P., Robbins, K., Mohle-Boetani, J. C., Vugia, D. J. 1999. Two outbreaks of multidrug-resistant *Salmonella* serotype Typhimurium DT104 infections linked to raw-milk cheese in Northern California. *JAMA*. vol. 281, no. 19, p. 1805-1810. <http://dx.doi.org/10.1001/jama.281.19.1805> PMID:10340367
- Claeys, W. L., Cardoen, S., Daube, G., De Block, J., Dewettinck, K., Dierick, K., et al. 2013. Raw or heated cow milk consumption: review of risks and benefits. *Food Control*, vol. 31, p. 251-262. <http://dx.doi.org/10.1016/j.foodcont.2012.09.035>
- Dahmen, S., Metayer, V., Gay, E., Madec, J. Y., Haenni, M. 2013. Characterization of extended-spectrum beta-lactamase (ESBL)-carrying plasmids and clones of *Enterobacteriaceae* causing cattle mastitis in France. *Vet. Microbiol.*, vol. 162, no. 2-4, p. 793-799. <http://dx.doi.org/10.1016/j.vetmic.2012.10.015> PMID:23127568
- De Buyser, M. L., Dufour, B., Maire, M., Lafarge, V. 2001. Implication of milk and milk products in food-borne diseases in France and in different industrialised countries. *Int. J. Food Microbiol.* vol. 67, p. 1-17. [http://dx.doi.org/10.1016/S0168-1605\(01\)00443-3](http://dx.doi.org/10.1016/S0168-1605(01)00443-3)
- Desmarchelier, P., Fegan, N. 2011. *Pathogens in milk: Escherichia coli*. Encyclopedia of dairy sciences, 2<sup>nd</sup> ed. Academic Press, London, p. 60-66. <http://dx.doi.org/10.1016/B978-0-12-374407-4.00393-9>
- Doumith, M., Buchrieser, C., Glaser, P., Jacquet, C., Martin, P. 2004. Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. *J. Clin. Microbiol.*, vol. 42, no. 8, p. 3819-3822. <http://dx.doi.org/10.1128/JCM.42.8.3819-3822.2004> PMID:15297538
- Fagan, P. K., Hornitzky, M. A., Bettelheim, K. A., Djordjevic, S. P. 1999. Detection of shiga-like toxin (*stx*<sub>1</sub> and *stx*<sub>2</sub>), intimin (*eaeA*), and enterohemorrhagic *Escherichia coli* (EHEC) hemolysin (EHEC *hlyA*) genes in animal feces by multiplex PCR. *App. Environ. Microbiol.*, vol. 65, p. 868-872. PMID:9925634
- Harrington, P., Archer, J., Davis, J. P., Croft, D. R., Varma, J. K., EIC officers. 2002. Outbreak of *Campylobacter jejuni* infections associated with drinking unpasteurized milk procured through a cow-leasing program-Wisconsin. *Morbidity and Mortality Weekly Report*. vol. 51, p. 548-549.
- Hata, E., Katsuda, K., Kobayashi, H., Nishimori, K., Uchida, I., Higashide, M., Ishikawa, E., Sasaki, T., Eguchi, M. 2008. Bacteriological characteristics of *Staphylococcus aureus* isolates from humans and bulk milk. *J. Dairy Sci.*, vol. 91, p. 564-569. <http://dx.doi.org/10.3168/jds.2007-0457> PMID:18218742
- Ikeda, T., Tamate, N., Yamaguchi, K., Makino, S. 2005. Mass outbreak of food poisoning disease caused by small amounts of staphylococcal enterotoxins A and H. *App. Environ. Microbiol.*, vol. 71, no. 5, p. 2793-2795. <http://dx.doi.org/10.1128/AEM.71.5.2793-2795.2005> PMID:15870376
- ISO 16649-1 Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli*. Part 1: Colony-count technique at 44 degrees C using membranes and 5-bromo-4-chloro-3-indolyl beta-D-glucuronide, 2012.
- ISO 6888-1 (560089) Microbiology of food and animal feeding stuffs, Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) Part 1: Technique using Baird-Parker agar medium, 2000.
- ISO 6579 (560088) Microbiology of food and animal feeding stuffs, Horizontal method for the detection of *Salmonella* spp., 2003.
- ISO 10272-1 (560126) Microbiology of food and animal feeding stuffs, Horizontal method for detection and enumeration of *Campylobacter* spp., Part 1: Detection method, 2006.
- ISO 11290-1 (560093) Microbiology of food and animal feeding stuffs, Horizontal method for the detection and enumeration of *Listeria monocytogenes*, Part 1: Detection method, 1999.
- Jørgensen, H. J., Mørk, T., Caugant, D. A., Kearns, A., Rørvik, L. M. 2005. Genetic variation among *Staphylococcus aureus* strains from Norwegian bulk milk. *App. Environ. Microbiol.*, vol. 71, no. 12, p. 8352-8361. <http://dx.doi.org/10.1128/AEM.71.12.8352-8361.2005> PMID:16332822
- Kalman, M., Szollosi, E., Czermann, B., Zimanyi, M., Szekeres, S. 2000. Milkborne campylobacter infection in Hungary. *J. Food Protection*. vol. 63, no. 10, p. 1426-1429. PMID:11041145
- Kathariou, S. 2002. *Listeria monocytogenes* virulence and pathogenicity, a food safety perspective. *J. Food Prot.* vol. 65, no. 11, p. 1811-1829. PMID:12430709
- Lee, J. H., Jeong, J. M., Park, Y. H., Choi, S. S., Kim, Y. H., Chae, J. S., Moon, J. S., Park, H., Kim, S., Eo, S. K. 2004. Evaluation of the methicillin-resistant *Staphylococcus aureus* (MRSA)-Screen latex agglutination test for detection of MRSA of animal origin. *J. Clin. Microbiol.*, vol. 42, no. 6,

p. 2780-2782. <http://dx.doi.org/10.1128/JCM.42.6.2780-2782.2004> PMID:15184469

Løvseth, A., Loncarevic, S., Berdal, K. G. 2004. Modified multiplex PCR method for detection of pryrogenic exotoxin genes in staphylococcal isolate. *J. Clin. Microbiol.*, vol. 42, no. 8, p. 3869-3872. <http://dx.doi.org/10.1128/JCM.42.8.3869-3872.2004> PMID:15297552

Lyra, D. G., Sousa, F. G., Borges, M. F., Givisiez, P. E., Queiroga, R. C., Souza, E. L., Gebreyes, W. A., Oliveira, C. J. 2013. Enterotoxin-encoding genes in *Staphylococcus* spp. from bulk goat milk. *Foodborne Pathog Dis.*, vol. 10, no. 2, p. 126-130. <http://dx.doi.org/10.1089/fpd.2012.1256> PMID:23441914

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*. *J. Clin. Microbiol.*, vol. 36, no. 3, p. 618-623. PMID:9508283

Martins, E. A., Leal Germano, P. M. 2011. *Listeria monocytogenes* in ready-to-eat, sliced, cooked ham and salami products, marketed in the city of São Paulo, Brazil: occurrence, quantification, and serotyping. *Food Control.*, vol. 22, no. 2, p. 297-302. <http://dx.doi.org/10.1016/j.foodcont.2010.07.026>

McCallum, N., Berger-Bachi, B., Senn, M. M. 2010. Regulation of antibiotic resistance in *Staphylococcus aureus*. *Int J Medical Microbiology*, vol. 300, p. 118-129. <http://dx.doi.org/10.1016/j.ijmm.2009.08.015>

Mork, T., Kvitle, B., Mathisen, T., Jorgensen, H. J. 2010. Bacteriological and molecular investigations of *Staphylococcus aureus* in dairy goats. *Vet. Microbiol.*, vol. 141, no. 1-2, p. 134-141. <http://dx.doi.org/10.1016/j.vetmic.2009.08.019> PMID:19733450

Oliver, S. P., Jayarao, B. M., Almeida, R. A. 2005. Foodborne pathogens in milk and the dairy farm environment: food safety and public health implications. *Foodborne Pathogens and Disease*, vol. 2, p. 115-129. <http://dx.doi.org/10.1089/fpd.2005.2.115>

Oliver, S. P., Boor, K. J., Murphy, S. C., Murinda, S. E. 2009. Food safety hazards associated with consumption of raw milk. *Foodborne Pathog Dis.*, vol. 6, no. 7, p. 793-806. <http://dx.doi.org/10.1089/fpd.2009.0302> PMID:19737059

Park, Y., W., Juárez, M., Ramos, M.m Haenlein, G. F. W. 2007. Physico-chemical characteristics of goat and sheep milk. *Small Ruminant Research*, vol. 68, p. 88-113. <http://dx.doi.org/10.1016/j.smallrumres.2006.09.013>

Poulsen, A. B., Skov, R., Pallesen, L. V. 2003. Detection of methicillin resistance in coagulase-negative staphylococci and in staphylococci directly from simulated blood cultures using the EVIGENE MRSA Detection Kit. *J. Antimicrob. Chemother.*, vol. 51, no. 2, p. 419-421. <http://dx.doi.org/10.1093/jac/dkg084> PMID:12562714

Robati, R., Gholami, S. 2013. Estimation of virulence genes of Shiga toxin producing *Escherichia coli* from juice puraches nd vegetables in Tehran/Iran. *Europ. J. Experimental Biology*, vol. 3, no. 2, p. 457-462. [cit. 2014-01-15]. Available at: <http://pelagiaresearchlibrary.com/european-journal-of-experimental-biology/vol3-iss2/EJEB-2013-3-2-457-462.pdf>

Scherrer, D., Corti, S., Muehlher, J. E., Zweifel, C., Stephan, R. 2004. Phenotypic and genotypic characteristics of *Staphylococcus aureus* isolates from raw bulk-tank samples of goat and sheep. *Vet. Microbiol.*, vol. 101, no. 2, p. 101-107.

<http://dx.doi.org/10.1016/j.vetmic.2004.03.016> PMID:15172692

Silva, E. R., Carmo, L. S. Silva, N. 2005. Detection of the enterotoxins A, B and C genes in *Staphylococcus aureus* from goat and bovine mastitis in Brazilian dairy herds. *Vet. Microbiol.* vol. 106, no. 1-2, p. 103-107. <http://dx.doi.org/10.1016/j.vetmic.2004.12.005> PMID:15737479

Šťástková, Z., Karpíšková, S., Karpíšková, R. 2009. Occurrence of methicillin-resistant strains of *Staphylococcus aureus* at a goat breeding farm, *Veterinarni Medicina*, vol. 54, p. 419-426. [cit. 2014-01-10]. Available at: <http://www.vri.cz/docs/vetmed/54-9-419.pdf>

Thomas, D., Chou, S., Dauwalder, O., Lina, G. 2007. Diversity in *Staphylococcus aureus* enterotoxins. *Chem. Immunol. Allergy*, vol. 93, p. 24-41. <http://dx.doi.org/10.1159/000100856> PMID:17369698

Vanderhaeghen, W., Cerpentier, T., Adriaensen, C., Vicca, J., Hermans, K., Butaye, P. 2010. Methicillin-resistant *Staphylococcus aureus* (MRSA) ST 398 associated with clinical and subclinical mastitis in Belgian. *Vet. Microbiol.*, vol. 144, no. 1-2, p. 166-171. <http://dx.doi.org/10.1016/j.vetmic.2009.12.044> PMID:20092969

Wani, S. A., Hussain, I., Fayaz, I., Mir, M. A., Nishikawa, Y. 2009. Subtype analysis of stx1, stx2 and eae genes in Shiga toxin-producing *Escherichia coli* (STEC) and typical and atypical enteropathogenic *E. coli* (EPEC) from lambs in India. *Veterinary Journal*, vol. 182, no. 3, p. 489-490. <http://dx.doi.org/10.1016/j.tvjl.2008.07.017> PMID:18768338

**Acknowledgment:**  
This study was financially supported by research project NAZV KUS QJ 1230044, QJ 1210300, QJ 1210284 and MEYS NPU program LO1218.

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