

## MICROBIOLOGICAL QUALITY AND SAFETY OF GOAT'S MILK FROM ONE FARM

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### Abstract

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In recent years, the popularity of goat's milk and goat's milk products has been growing in the Czech Republic, especially for its low allergenic potential and good digestibility. This study focused on the assessment of the microbiological quality and safety of raw and heat-treated goat's milk. During the lactation period, total of 48 samples of raw and 40 samples of pasteurized goat's milk were collected on a goat's farm in the South Moravian Region of the Czech Republic. Quantitative analysis was performed to determine the total plate count (TPC) and coagulase-positive (CP) staphylococci count. The presence of *E. coli* including *E. coli* O157, CP staphylococci, *B. cereus*, *L. monocytogenes*, *Salmonella* spp., and *Campylobacter* spp. was detected. The monthly average TPC ranged from 4.53 to 5.21 log CFU.ml<sup>-1</sup> in raw milk and from 2.36 to 3.71 log CFU.ml<sup>-1</sup> in pasteurized milk. Thirty (75.0%) *S. aureus* isolates from raw milk carried the *sec* gene, two (5.0%) were positive for the genes *seb*, *seg*, *sei*, and one (2.5%) harboured the *seg* and *sei* genes. Pasteurized goat's milk samples yielded a single isolate of *S. aureus* carrying the *sec* gene. One isolate of *E. coli* serotype O156 producing ST1 toxin was recovered from raw milk. *B. cereus* was detected only in two pasteurized goat's milk samples. Any other pathogens monitored were not detected. In this study, shigatoxin-producing *E. coli* O156 was detected in raw goat's milk for the first time in the Czech Republic.

raw milk, pasteurized milk, total plate count, staphylococcal enterotoxins, STEC, indicator bacteria, pathogens

In 2005, 13 000 goats were registered in the Czech Republic, out of which 386, i.e. 3.2%, were in the South Moravian Region. In total, 1,100,000 litres of goat's milk and 110 tonnes of goat's cheeses were produced in this country. In the whole of the Czech Republic, there are now 19 farms with more than 50 animals. One of these farms was monitored in this study. It is located in the Blansko District of the South Moravian Region (Bucek *et al.*, 2007).

Goat's milk as compared to cow's milk has better digestibility (Park, 2000), higher alkalinity (Aganga *et al.*, 2002) and a higher buffering capacity. They also differ in the contents of basic milk components (Park, 2000). Raw goat's milk has specific sensory properties – its white colouring is due to the absence of carotene and its specific smell and taste arise from the content of free fatty acids. Goat's milk is less allergenic than cow's milk (Martín-Diana *et al.*, 2003).

Goat's milk can be, just as cow's or sheep's milk, a source of technologically undesirable or even pathogenic bacteria: *Listeria monocytogenes* (Gaya *et al.*, 1996; Abou-Eleinin *et al.*, 2000), shigatoxin-producing *Escherichia coli* (STEC), enterotoxin-producing *Staphylococcus aureus* (Foschino *et al.*, 2002; Muehlherr *et al.*, 2003), *Campylobacter* spp., and *Yersinia enterocolitica* (Roberts, 1985). To a lower degree, *Bacillus cereus* can also be found in goat's milk and cheeses (Papageorgiou *et al.*, 1998; Meena *et al.*, 2000).

Microbiological quality of the goat milk from Czech dairy farms has received relatively little attention to date. Given the increased demand for goat milk and goat milk products, this issue has recently come to the forefront of concern. A better knowledge of the microbiological quality of the goat milk will contribute to further research aimed at the

improvement of the quality of raw and pasteurized goat milk. This study focused on 1) the assessment of the microbiological quality of raw and heat-treated goat's milk during the lactation period, 2) the compliance of the results with the EU limits and 3) the assessment of health risk to humans from the consumption of raw goat's milk.

## MATERIALS AND METHODS

### Milk samples

Sample collection was carried out on a goat's farm in the South Moravian Region, Czech Republic, during lactation period after weaning the kids at regular intervals: total of 48 samples of raw goat's milk and 40 samples of pasteurized goat's milk were obtained. On the farm, there were 75 goats of the white short-haired breed in the 1<sup>st</sup> to 8<sup>th</sup> lactation. The average daily milk yield is 2–3 l and the average annual milk yield is 600–800 litres.

### Milk processing

Goats are machine milked twice daily. A thorough pre-milking semi-dry udder cleaning is carried out. The milking takes place in the designated area of the stable using a pipe milking machine. Milking machine sanitation is performed in a closed circuit way using approved sanitation products. After milking, the milk is cooled down promptly to 4–6 °C and then stored for 12–24 hours until further processing, i.e. stationary pasteurization in a tank at 72 °C for 20 seconds. The pasteurized milk is a semi-product for the production of fresh cheese in various flavours.

### Sampling

Raw milk samples were collected after cooling at 4–6 °C and pasteurized milk samples were collected after the heat treatment and subsequent cooling at 4–6 °C. The samples were transported to the laboratory at a maximum temperature of 8 °C and processed. The initial sample processing was performed according to the ČSN ISO 7218 (1998) guidelines.

### Quantitative microbiological analysis

In both raw and pasteurized goat's milk samples, the following indicators were assessed:

- total plate count (TPC): 1 ml of sample was inoculated onto Plate Count Agar (Oxoid, UK) (ČSN ISO 4833, 2003),
- coagulase-positive staphylococci count: 0.2 ml of sample was inoculated onto Baird-Parker Agar (Oxoid, UK) (ČSN EN ISO 6888-1, 1999).

### Qualitative microbiological analysis

Every assessment, with the exception of *Campylobacter* spp. and *Listeria monocytogenes*, started with aerobic culture of the sample in buffered peptone water (Oxoid, UK) at 37 °C for 18–20 hours.

The below-mentioned qualitative parameters were assessed in the samples of raw and pasteurized goat's milk:

- *E. coli* detection: the pre-enriched sample was inoculated onto Tryptone Bile X-Glucuronide Medium (Bio-Rad, France) to be cultured aerobically at 44 °C for 18–20 hours,
- *E. coli* O157 detection: the pre-enriched sample was concentrated by immunomagnetic separation (Dynabeads® anti-*E. coli* O157, Invitrogen Dynal AS, Norway) and then spread onto Sorbitol Mac Conkey Agar with BCIG (Oxoid, UK) to be cultured aerobically at 37 °C for 24 hours (ČSN EN ISO 16654, 2002),
- coagulase-positive staphylococci detection: the pre-enriched sample was inoculated onto Baird-Parker Agar (Oxoid, UK) to be cultured aerobically at 37 °C for 48 hours,
- *Bacillus cereus* detection: the pre-enriched sample was inoculated onto Mannitol Yolk Polymyxin B Agar (Oxoid, UK) to be cultured aerobically at 30 °C for 48 hours,
- *Listeria monocytogenes* detection: was performed according to ČSN EN ISO 11290-1 (1999) using the Half Fraser broth, Fraser broth and ALOA agar provided by Bio-Rad (France),
- *Salmonella* spp. detection: was performed according to ČSN EN ISO 6579 (2003) using buffered peptone water (non-selective enrichment), RVS medium and MKTTn medium (selective enrichment), and Xylose Lysine Deoxycholate Agar provided by Oxoid (UK).

In raw milk samples, thermotolerant *Campylobacter* spp. were also monitored. Ten millilitres of raw milk were aseptically concentrated by centrifugation at 4 000 rpm for 5 minutes. The sediment was spread on the surface of modified Charcoal Cefoperazone Deoxycholate Agar (Oxoid, UK) with a sterile cotton swab. The inoculated plates were incubated under micro-aerophilic conditions at 42 °C for 48 hours. The confirmation of suspected *Campylobacter* colonies was carried out according to ČSN EN ISO 10272-1 (1999).

### Confirmation of *S. aureus* isolates and enterotoxin detection

To confirm *S. aureus*, 2–4 suspected colonies were taken from the selective medium for each milk sample. The coagulase test was performed according to ČSN EN ISO 6888-1 (1999). PCR was used to detect the SA442 species-specific DNA fragment (Martineau *et al.*, 1998) and the genes encoding enterotoxin production (*sea – sej*) (Monday and Bohach, 1999; Løvseth *et al.*, 2004). At the same time, RPLA (SET-RPLA, Denka Seiken, Japan) was used to check the capability of the *S. aureus* isolates to produce A–D enterotoxins.

### Statistical analysis

When calculating the average monthly values of the quantitative parameters, the data were checked

with Grubbs' test for extreme outliers to prevent them from being involved (UNISTAT software, version 5.1, UNISTAT Ltd., UK).

## RESULTS AND DISCUSSION

### Quantitative analysis

The average monthly total plate counts (TPC) and coagulase-positive staphylococci counts in raw and pasteurized goat's milk are presented in Fig. 1.

For raw goat's milk, the limit value of the rolling geometric average of TPC over a two-month period, with at least two samples per month, is set at  $\leq 1.5 \cdot 10^6$  CFU.ml<sup>-1</sup> (i.e. 6.18 log CFU.ml<sup>-1</sup>) according to Commission Regulation (EC) No 1662/2006. As becomes clear from Fig. 1, over the whole of the period, this limit was not exceeded. The average monthly TPC in raw goat's milk ranged from 4.53 to 5.21 log CFU.ml<sup>-1</sup>, peaking in July. These results correspond to the findings of other authors (Foschino *et al.*, 2002) who assessed raw goat's milk from farms in the Bergamo area in Italy. They have reported an average TPC of 4.70 log CFU.ml<sup>-1</sup>. In Swiss farms with more than 25 goats, the average TPC in raw goat's milk was 4.97 log CFU.ml<sup>-1</sup> (Muehlherr *et al.*, 2003).

In pasteurized milk, TPC ranged from 2.36 to 3.71 log CFU.ml<sup>-1</sup>. A significant decrease in TPC of pasteurized milk was observed in the period from September to October. After consulting with the owner, we found out that early in September, the control of the pasteurization unit was adjusted which had its effect in a TPC drop.

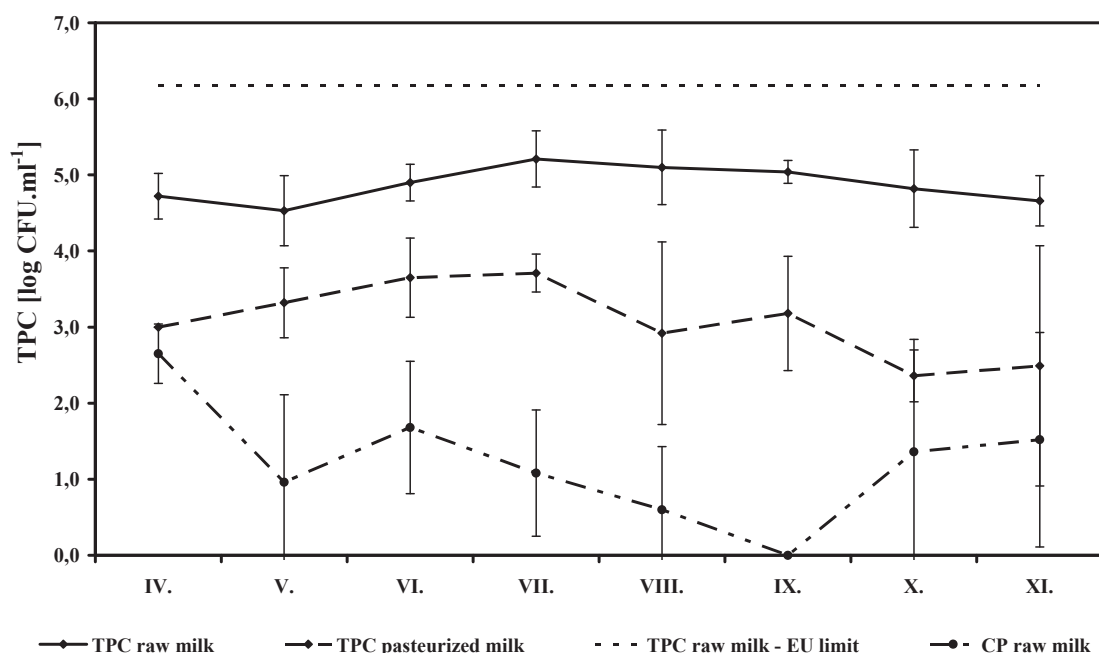
The average monthly coagulase-positive staphylococci (CP) counts ranged significantly from  $< 1.69$  log CFU.ml<sup>-1</sup> (i.e.  $< 50$  CFU.ml<sup>-1</sup>) to 2.65 log CFU.ml<sup>-1</sup> in raw goat's milk and  $< 1.69$  log CFU.ml<sup>-1</sup> in pasteurized milk. A similar incidence of *S. aureus* has also been reported by Muehlherr *et al.* (2003). While analysing 344 samples of raw goat's milk, they established the median *S. aureus* count to be  $< 1.0$  log CFU.ml<sup>-1</sup>. On the other hand, their peak *S. aureus* count of 4.34 log CFU.ml<sup>-1</sup> was significantly higher than the peak of 2.61 log CFU.ml<sup>-1</sup> in our study.

### Qualitative analysis

*S. aureus* was detected in 33(68.8%) of 48 raw goat's milk samples analyzed. In total, 40 *S. aureus* strains were isolated from raw goat's milk. Among these 40 *S. aureus* isolates, 33 strains were carriers of genes encoding enterotoxin production: 30 strains (75.0%) carried *sec*, 2 strains (5.0%) were positive for *seb*, *seg*, and *sei*, and 1 strain (2.5%) harboured *seg* and *sei*. Pasteurized goat milk yielded a single *S. aureus* isolate after enrichment. It carried the *sec* gene. The RPLA method confirmed the ability of the *S. aureus* isolates carrying the *seb* and *sec* genes to produce enterotoxins B and C.

The occurrence of *S. aureus* in raw goat's milk can vary widely depending on local farm conditions. This hypothesis is supported by Vyleťlová *et al.* (2011) who have reported the detection of *S. aureus* in 17% only of raw milk samples collected on a farm in North Moravia from the same breed of goats as used in our study.

*S. aureus* isolates from goat's milk are often carriers of the *sec* gene (Foschino *et al.*, 2002; Orden *et al.*, 1992). It is in accordance with our results as the



Notes: TPC – total plate count, CP – coagulase-positive staphylococci count

1: The trends in TPC and coagulase-positive staphylococci count in raw and pasteurized goat's milk (monthly mean  $\pm$  SD)

*sec* gene was detected in 75.0% of *S. aureus* isolates. This enterotoxin is dominant in *S. aureus* strains recovered from mastitis infected milk. Genes responsible for enterotoxin production were detected in 61.8% of 262 *S. aureus* isolates (Scherrer *et al.*, 2004). In their isolates, *sec* was most often detected (35.9%), followed by *seg* (11.8%), *sea* (10.7%), *sej* (9.9%), *sei* (9.1%), *seb* and *sed* (1.5 %). With regard to the number of the samples analyzed and to the aim to analyze milk from one farm, our *S. aureus* isolates did not display such a wide range of genes as those in the above-mentioned study.

The coagulase-positive staphylococci counts detected in raw milk ( $< 1.69 \log \text{CFU.ml}^{-1}$  to  $2.65 \log \text{CFU.ml}^{-1}$ ) did not reach  $5.00 \log \text{CFU.ml}^{-1}$ , i.e. the amount of bacteria necessary for the production of an enterotoxin dose capable of inducing food-borne intoxication (Ercolini *et al.*, 2004). Nevertheless, when raw milk is stored under poor conditions or when pasteurized milk used for fresh cheese production is exposed to secondary contamination, *S. aureus* can propagate in it, producing heat-stable enterotoxins (Balaban and Rasooly, 2000).

A  $\beta$ -D-glucuronidase-positive *E. coli* serotype O156 isolate producing ST1 toxin was recovered from a raw milk sample. STEC of serogroup O156 have been reported in ruminants, although not as the dominant serotype. Beutin *et al.* (1993), e.g., have isolated two *E. coli* strains of serotype O156:H21 from cattle, one of which produced ST1 toxin and the other both ST1 and ST2. Also Blanco *et al.* (2004) have isolated 514 STEC strains from cattle, 11 (2.14%) of which belonged to serogroup O156. Four of the above-mentioned strains carried the *stx1* gene and seven of these strains were carriers of the *stx2* gene. In Spain, apart from cattle, STEC O156 strains were isolated also from rectal swabs from sheep and goats (Blanco *et al.*, 2003; Cortés *et al.*, 2005). Similarly, Muehlherr *et al.* (2003) have isolated 12 STEC strains belonging to the non-O157 group from goat's milk; nevertheless, no serotype O156 isolate was detected.

Based on our results, it can be concluded that even Czech goats can be a reservoir of non-O157 serotypes of STEC. The consumption of raw goat's milk or goat's milk products can pose health risk to consumers, especially in the light of the fact that the goat's milk is recommended for children allergic to cow's milk and also for persons with decreased immunity. A case report has already been presented of *E. coli* O157 infection in a consumer of unpasteurized goat's milk (Bielaszewska *et al.*, 1997). However, the adequate heat treatment of milk (at 72 °C for 15 s, as laid down by Commission Regulation (EC) No. 1662/2006), kills STEC in it.

*Salmonella* spp., *Campylobacter* spp. and *Listeria monocytogenes* were not isolated from either raw or pasteurized milk. *Bacillus cereus* was only detected in two pasteurized goat's milk samples (5.0%), both of them collected in October. The absence of *Salmonella* in raw goat's milk has also been pointed out by others (Foschino *et al.*, 2002; Muehlherr *et al.*, 2003). Low-level detection of *Campylobacter* spp. bacteria in raw goat's milk has been presented by Muehlherr *et al.* (2003). On the other hand, Harris *et al.* (1987) have reported six cases of campylobacteriosis in King County in the US state of Washington linked to the consumption of raw goat's milk. Low levels of *Listeria* spp. in raw goat's milk have been detected by many authors (Gaya *et al.*, 1996; Abou-Eleinin *et al.*, 2000).

## CONCLUSIONS

In summary, it can be concluded that the raw goat's milk from this farm is in compliance with the EU limits for goat's milk. Apart from STEC O156 and enterotoxin-producing *S. aureus*, no pathogenic microorganisms were found in either raw or in pasteurized goat's milk. The consumption of pasteurized goat's milk and goat's milk products from this farm might pose low health risk to consumers.

## SUMMARY

This study focused on the assessment of the microbiological quality and safety of raw and heat-treated goat's milk during the lactation period, on the comparison of the results with the EU limits, and on the assessment of health risks to consumers. Forty-eight samples of raw goat's milk and forty samples of pasteurized goat's milk were collected during the lactation period on a goat's farm in the Blansko district of the South Moravian Region, the Czech Republic. The following microbiological characteristics were determined: total plate count (TPC), detection and count of coagulase-positive staphylococci, detection of *E. coli* including *E. coli* O157, *B. cereus*, *L. monocytogenes*, *Salmonella* spp., and heat-tolerant *Campylobacter* spp. PCR was used to identify *S. aureus* and to detect enterotoxin-specific genes (*sea-sej*). The RPLA method was used to check the capability of the *S. aureus* isolates to produce A–D enterotoxins. When calculating the average monthly values, the data were checked with Grubbs' test for extreme outliers. The average monthly TPC in raw goat's milk ranged from 4.53 to  $5.21 \log \text{CFU.ml}^{-1}$ , peaking in July. In pasteurized milk, TPC varied from 2.36 to  $3.71 \log \text{CFU.ml}^{-1}$ . The average monthly coagulase-positive staphylococci counts ranged significantly from  $< 1.69 \log \text{CFU.ml}^{-1}$  (i.e.  $< 50 \text{CFU.ml}^{-1}$ ) to  $2.65 \log \text{CFU.ml}^{-1}$  in raw goat's milk, and  $< 1.69 \log \text{CFU.ml}^{-1}$  in pasteurized goat's milk. In 40 *S. aureus* isolates from raw milk, genes encoding enterotoxin production were detected: 30 (75.0%) isolates carried the *sec* gene, two (5.0%) isolates were positive for the *seb*, *seg*, and *sei* genes and one (2.5%) isolate was the carrier of the *seg* and *sei* genes. Pasteurized goat's milk yielded a single isolate



of *S. aureus* carrying the *sec* gene. One *E. coli* serotype O156 isolate producing VT1 toxin was recovered from raw goat's milk. *Salmonella* spp., *Campylobacter* spp., and *L. monocytogenes* were not isolated from either raw or pasteurized milk. *B. cereus* was only detected in two pasteurized goat's milk samples (5.0 %). In this study, shigatoxin-producing *E. coli* O156 was detected in raw goat's milk for the first time in the Czech Republic.

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