The microbiological safety of goat colostrum in the Czech Republic

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Abstract

A total of 460 raw goat colostrum samples (424 native, 36 lyophilised) from Czech ecological farms were analysed during the years 2012 – 2015. The aim of this study was to determine the microbial safety of goat colostrum in the Czech Republic (the presence of *Listeria monocytogenes*, *Salmonella* spp., Shiga toxin-producing *Escherichia coli* (STEC), methicillin-resistant *S. aureus* (MRSA) and *S. aureus* with genes encoding the production of staphylococcal enterotoxins). *Listeria monocytogenes* was detected in 28 samples (6.1%), potentially pathogenic *Escherichia coli* in 12 samples (2.6%) and *Staphylococcus aureus* in 84 samples (18.3%) in goat colostrum.

Colostrum, enterotoxins, ESBL, Listeria monocytogenes, MRSA, Salmonella spp.

Introduction

Colostrum is the product of the mammary gland secretion of mammals produced shortly before and after the birth of a baby (Gauthier et al. 2006). The microbiological quality of raw colostrum varies considerably. Microbial contamination occurs most frequently during milking and storage, but also as a result of mastitis, feeding or bacterial proliferation (Johnson et al. 2007). Colostrum is an important source of many nutrients and bioactive substances which are necessary not only for babies but also for the young. Pathogenic agents may be a risk factor affecting the safety of colostrum. This topic is of great concern because the microorganisms present in colostrum may cause serious diseases (Godden 2008). Houser et al. (2008) state that, the greatest detection of microorganisms is observed in the presence of aerobic mesophilic microorganisms, can also occur to the present of pathogenic strains, such as *Salmonella* spp., pathogenic strains of *Escherichia coli*, *Mycobacterium avium* ssp. *paratuberculosis* and *Mycoplasma* spp. This fact should be taken into consideration in relation to the processing of colostrum for human consumption. On the basis of this fact, this study is focused on mapping the occurrence of bacteriological risks in goat colostrum.

Materials and Methods

A total of 460 raw goat colostrum samples (424 native, 36 lyophilised) from ecological farms in the Czech Republic were analysed in the years 2012 – 2015. Samples were processed and analysed immediately following the delivery to the laboratory. The farms were monitored for the hygiene quality of goat colostrum (*Listeria monocytogenes*, *Salmonella* spp., Shiga toxin-producing *Escherichia coli* (STEC), methicillin-resistant *S. aureus* (MRSA) and *S. aureus* with genes encoding the production of staphylococcal enterotoxins).

The presence of *Listeria monocytogenes* was determined according to CSN EN ISO 11290-1, primary enrichment took place in half Fraser broth (OXOID, UK) at 30 °C for 24 hours, and was followed by inoculation on full Fraser broth (OXOID, UK) at 37 °C for 24 – 48 hours. Inoculation was performed on ALOA agar (BIORAD, France) and cultivation was performed at 37 °C for 24 – 48 hours. Colonies typical for *Listeria monocytogenes* were confirmed and serotyped by slide agglutination using commercially available antisera (Denka Seiken, Japan) and verified by multiplex PCR (Doumith et al. 2004).

Detection of *Salmonella* spp. was performed according to CSN EN ISO 6579. After enrichment in peptone water (OXOID, UK), selective enrichment in RVS and MKTTN media (OXOID, UK) was performed. This was followed by inoculation on RAMBACH (Merck, Germany) and XLD (OXOID, UK) agars.

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Detection of *E. coli* was performed according to CSN EN ISO 16649-1 using a method known as the horizontal method for the determination of β-glucuronidase-positive *Escherichia coli* by means of the technique of counting colonies cultured at 44 °C using 5-bromo-4-chloro-3-indolyl β-D-glucuronide. The detection was performed by a modification of the CSN ISO 16649-2 method after sample enrichment in buffered peptone water (OXOID, UK) at 37 °C for 24 hours with subsequent cultivation on TBX agar (44 °C, 24 hours). Confirmation of suspect isolates consisted of a negative oxidase reaction (OXItest, ERBA-LACHEMA, Czech Republic) and positive indole reaction (COLItest, ERBA-LACHEMA, Czech Republic). The presence of selected virulence factors was monitored in *E. coli* strains. Multiplex PCR according to Fagan et al. (1999) was used for the detection of genes encoding selected virulence factors *eae*A (intimin), *hly* (haemolysin), stx_1 and stx_2 (verotoxin 1 and 2). Multiplex PCR for the detection of genes encoding resistance to β-lactams bla_{TEM}, bla_{SHV} and $bla_{CTX\text{-}m}$ was also performed (Briñas et al. 2002; Lewis et al. 2007).

Detection of coagulase-positive staphylococci was performed according to CSN EN ISO 6888-1. Detection was performed after propagation in peptone water (OXOID, UK). Baird-Parker Medium (OXOID, UK) was used for cultivation. The identification of suspect colonies was performed by the detection of coagulase (DENKA SEIKEN, Japan). Confirmation of suspect strains of *S. aureus* was performed by a polymerase chain reaction with specific SA442 fragment detection (Martineau et al. 1998). PCR for the detection of the *mec*A gene, which is responsible for resistance to methicillin, was performed for the determination of MRSA in *S. aureus* isolates (Poulsen et al. 2003). Detection of genes encoding enterotoxins SEA-SEE and SEH was performed by multiplex PCR according to Løvseth et al. (2004).

Results and Discussion

This study was focused to map the occurrence of bacteriological risks in goat colostrum. While some colostrum was bacteriological safe in other colostrum samples pathogenic microorganisms were detected. In our study, *Listeria monocytogenes*, *Escherichia coli* and *Staphylococcus aureus* were detected.

Prevalence of *Listeria monocytogenes* in colostrum

Listeria monocytogenes was detected in 28 samples (6.1%; 22 native and 6 lyophilised), isolates of serotypes 1/2a and 4b were detected in the colostrum samples. In previous studies in other countries, serotypes 1/2a, 1/2b and 4b were frequently detected from dairy farms (Fedio and Jackson 1992; Van Kessel et al. 2004; Borucki et al. 2005). Our results are in agreement with these findings. Our results suggest that isolates from colostrum have the potential to cause human and animal listeriosis.

Prevalence of *Salmonella* spp. in colostrum

Salmonella spp. was not detected in the tested colostrum samples.

Prevalence of *Escherichia coli* in colostrum

In the past few years, there has been growing concern in the scientific community about the emergence and dissemination of *E. coli* isolates producing ESBL being very frequently associated with alimentary infections (Pitout et al. 2004).

In our study, potentially pathogenic *E. coli* was detected in 12 samples (2.6%; in native colostrum only). Three colonies of *E. coli* were isolated from each sample, for further testing only isolates with different resistance phenotypes from one sample were included in the study. Some of the virulence factors were detected in the tested samples. The stx_1 gene was present in 6 samples (1.3%), the *eae* gene was present in 5 samples (1.1%) and the *eae, hly* gene was detected in one isolate. The low prevalence of *eae* positivity (1.1%) indicates the absence of a locus for enterocyte attaching and effacing lesions. Other toxins such as *hly* present on the large plasmid may also contribute to the severity of STEC illness (Kumar et al. 2014). The genes encoding resistance to β-lactams we not detected in the tested strains of *E. coli*.

Prevalence of *Staphylococcus aureus* in colostrum

When investigating the incidence of pathogenic microorganisms, the highest detection rate was recorded for *Staphylococcus aureus*. A total of 84 isolates of *S. aureus* (18.3%) were obtained for further detection of enterotoxin-encoding genes. Two colonies of *S. aureus* were isolated

from each sample, for further testing only isolates with different phenotypes were included in the study. Staphylococcal enterotoxin outbreak has always been threat to dairy farms and the frequent reports of raw milk products being contaminated with various types of *S. aureus* clearly demonstrate the significance of this pathogen (Schønberg and Wåltorp 2001). In terms of the risk of foodborne diseases, the ability of approximately 50 – 75% of *S. aureus* strains to produce extracellular thermostable enterotoxins (SEs) under suitable conditions is a particular problem (Argudín et al. 2010). A total of 58 of these isolates (69.0%) were positive for the production of classical SEA-SEE enterotoxins which are the leading cause of foodborne intoxication. The enterotoxins SEB and SEC were detected most frequently.

The occurrence of pathogenic microorganisms resistant to routinely used antibiotics is becoming a global problem of the 21st century. In recent years, the increase in staphylococci strains that show resistance to methicillin/oxacillin has become a serious clinical and epidemiological problem. MRSA strains harbour the *mec*A gene which encodes a modified PBP2 protein with a low affinity for methicillin and all β-lactam antibiotics (Velasco et al. 2005). MRSA were not detected in our study.

Conclusions

The results of this study confirm the presence of pathogenic bacteria in native and lyophilised goat colostrum. These bacteria can be an important source of foodborne diseases, which could be a risk for immunocompromised persons. Lyophilisation does not eliminate *L. monocytogenes* totally. Consumption of goat colostrum is unsafe for high-risk groups as small children, pregnant women and older people.

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