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# Foodborne pathogens in unpasteurized milk in Sweden

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ABSTRACT

Raw milk may be a risk for public health if it is contaminated with zoonotic pathogens. To study the prevalence in unpasteurized milk from Swedish farms, bovine and small ruminant dairy farms were sampled. Since the sampling method and transport conditions may influence the outcome of analyses, efforts were made to optimize the methodology. Culturing of bacteria was done from in-line milk filters collected from the milk pipe at the point where it enters the milk bulk tank at the farms and this way of sampling was compared to sampling bulk tank milk (BTM) directly. Analysing milk filters were found to be superior to analysing BTM directly. Conditions for transport of milk filter samples were further improved by the addition of Cary Blair transport medium, which significantly increased the number of positive samples for pathogenic bacteria. The isolation of several foodborne pathogens from milk filters was demonstrated. The prevalence of samples with *Staphylococcus aureus* was 71% and 64%, and *Listeria* spp. 21% and 29% from dairy cow and goat/sheep farms, respectively. *Campylobacter jejuni*, *Yersinia enterocolitica* and verotoxigenic *Escherichia coli* (VTEC) O157 were detected in 9%, 2% and 2% of samples from bovine milk, respectively.

We conclude that the choice of sampling method and sample handling influence the results of bacterial culturing. From the results of this study, we strongly recommend to sample in-line milk filters instead of BTM directly and to use Cary Blair medium during transport, especially if the samples are to be analysed for *Campylobacter* spp. and/or *Listeria* spp. The findings also show that unpasteurized milk from Swedish farms occasionally contain bacteria with zoonotic potential.

## 1. Introduction

There is an increasing demand for unpasteurized milk, also known as raw milk, from consumers. The popularity has been explained by the assumption that raw milk provides better nutritional quality and a beneficial microflora as well as reduced risk for lactose intolerance, allergy and asthma. The scientific evidence for these claims is however weak (Lucey, 2015). On the contrary, raw milk may be a risk for public health if contaminated with zoonotic pathogens such as *Campylobacter*, *Salmonella*, *Yersinia*, *Listeria*, verotoxigenic *Escherichia* (*E.*) *coli* (VTEC) O157 and *Staphylococcus* (*S.*) *aureus*. These pathogens are often part of the intestinal flora or present on the udder of healthy dairy animals and can easily contaminate the environment and the milk during the production process (Gopal et al., 2015). Pasteurization is an effective way to improve milk safety by destroying pathogenic microorganisms that may occur in the milk (Lucey, 2015). When unpasteurized milk is used for cheese making, potentially pathogenic bacteria are expected to be killed during the fermentation process. However sometimes this does not work properly and/or the cheese becomes recontaminated during manufacturing, which may lead to outbreaks of foodborne illness.

*Campylobacter*, *Salmonella*, *Yersinia*, and VTEC O157 cause gastrointestinal disease with diarrhoea, fever and nausea as major symptoms in humans. Severe complications and sequelae sometimes occur, i.e. bacteremia, reactive arthritis, irritable bowel syndrome, and Guillain Barré syndrome. In the case of VTEC infection, it is assumed that up to 10% of cases develop haemolytic uremic syndrome (HUS), a potentially life-threatening condition, especially in children and elderly people (Mead and Griffin, 1998). *Listeria* (*L.*) *monocytogenes* usually causes fever and muscle pain and sometimes gastrointestinal symptoms. However, in individuals at high risk, e.g. pregnant women, the elderly and immunocompromised persons, the infection can be very serious with high mortality (Camargo et al., 2016). Other bacteria, such as *S.*

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*aureus* can produce enterotoxins, causing gastrointestinal disorders with vomiting, stomach pain and diarrhoea. Within EU, staphylococcal enterotoxins (SEs) are thought to be involved in up to 10% of all food poisoning outbreaks (EFSA, 2016). Dairy products are among the most commonly reported products behind these outbreaks. Enterotoxins can be produced by *S. aureus* under favourable conditions such as storage of unchilled milk, but also in cheese made from unpasteurized milk or after recontamination during processing (Hennekinne et al., 2012).

Outbreaks or sporadic disease associated with raw milk or cheese made of raw milk are increasingly being reported especially in Western countries (EFSA, 2016; Giacometti et al., 2012; Johler et al., 2015; Lahti et al., 2017; Longenberger et al., 2014; Ruusunen et al., 2013). In Sweden, outbreaks associated with drinking raw milk, most often caused by *Campylobacter* or VTEC, have been reported several times. In many cases, visitors to farms have become ill after they have been offered and consumed unpasteurized milk (Anon., 2012). As reviewed by Oliver et al. (2005) the prevalence of pathogens in bulk tank milk varies between studies. *Campylobacter* was found in 0.4–12.3%, shiga-toxin producing *E. coli* in 0.8–3.8%, *L. monocytogenes* in 1.0–12.6% and *Salmonella* spp. in 0.2–8.9% of bulk tank milk samples in different surveys. Several factors influence the prevalence, e.g. farm size, number of animals on the farm, milking hygiene, farm management and season. However, technical details concerning sampling methods, transport conditions and analytical methods also have a crucial impact on the outcome of the laboratory results. Thus, the optimization of the screening process for the studied pathogens is essential.

The aim of the study was to determine the prevalence of 6 different zoonotic foodborne bacteria in unpasteurized milk from primary dairy production at Swedish farms and to compare sample types and conditions for transport of the samples to the laboratory.

## 2. Material and methods

This work was performed in two parts, named the initial study (sampling period 1 and 2) and the additional study (Table 1).

### 2.1. Sampling

#### 2.1.1. Initial study

In the initial study, 82 farmers with dairy cows and 17 with dairy goats/sheep were contacted and asked to join the study. Farmers were selected either because they delivered milk to a small-scale dairy or via their dairy extension service veterinarian and farms were chosen to represent different geographical areas of Sweden. Samples were collected during two periods, September to November 2011 (sampling period 1), and June to August 2012 (sampling period 2).

*2.1.1.1. Sampling period 1.* A sampling kit was sent to the farmers including a remittance form, sampling instructions, gloves, plastic bags and an ice pack. For the dairy cow farms information about herd size, breed, housing system and the latest recorded bulk tank milk somatic cell count (SCC), as well as the bulk tank milk bacterial count was requested. For the goat/sheep farms, information regarding herd size and housing system was requested. All farms were also asked to report whether they had an on-farm dairy and/or if the milk from the farm was used to produce unpasteurized products.

The farmers were asked to collect the milk filter, located in the milk pipe at the point where it enters the bulk milk tank, after milking. The filter was placed in a plastic bag and sent together with an ice pack, to keep the sample chilled during the transport to the laboratory at the National Veterinary Institute (SVA) in Uppsala, Sweden.

*2.1.1.2. Sampling period 2.* Sampling was performed as in sampling period 1, with the modification of including 25 mL of Cary Blair transport medium (Difco Becton Dickson, Sparks, MD, USA and Merck, Whitehouse Station, NJ, USA) in the sampling kit. The farmer was instructed to add the medium to the milk filter in the plastic bag immediately after collection of the milk filter, after which the filter with the medium was transported to the laboratory.

#### 2.1.2. Additional study

The additional study was performed in May to June 2014 to evaluate the sensitivity of milk filters testing in comparison with sampling directly from the bulk tank milk (BTM), for detection of thermophilic *Campylobacter* spp. and *Listeria* spp. Fifteen dairy cow farms, which all had delivered positive samples for at least one pathogen other than *S. aureus* in the initial study, were asked to send in additional samples. The same sampling procedure as the one in sampling period 2 of the initial study was used. In addition, a 50 mL sterile test tube for the collection of BTM was sent to the farmers. Both types of samples were collected at the same time and in the laboratory, both types of samples were examined in parallel.

### 2.2. Handling and laboratory analyses of samples

Only samples that reached the laboratory within 24–48 h were included in the study. Standardised reference methods, i.e. ISO or NMKL methods were applied as indicated. Analysis for *Salmonella* spp. was only performed if the farmer had given written consent to this analysis. All buffers and media used were prepared at SVA.

At the laboratory, the milk filters were transferred to stomacher bags, and in sampling period 1, 10 mL of Buffered Peptone Water (BPW; Oxoid, Basingstoke, Hampshire, UK) was added to the stomacher bags. The milk filters were processed in a stomacher for 1 min and aliquoted

**Table 1**
Handling of samples (numbers in parenthesis) in the initial and additional study, respectively, for analysis of zoonotic pathogens in raw milk after different transport conditions, as indicated.

| | Initial study | | Additional study | |
|---|---|---|---|---|
| | Sampling period 1 | Sampling period 2 | | |
| Sample type | Milk filters (51) without transport medium | Milk filters (52) with 25 mL Cary Blair transport medium | Milk filters (15) with 25 mL Cary Blair transport medium | Bulk tank milk samples (15) without transport medium |
| Preparation at the laboratory before analysis | Milk filter stomached in 10 mL Buffered Peptone Water | Milk filter stomached in the Cary Blair medium | Milk filter stomached in the Cary Blair medium | No preparation of bulk tank milk before enrichment |
| Analysis for | *Campylobacter* spp.<br>*VTEC* O157<br>*Salmonella* spp.<br>(n = 32)*<br>*Listeria* spp.<br>*Yersinia enterocolitica*<br>*Staphylococcus aureus* | *Campylobacter* spp.<br>*VTEC* O157<br>*Salmonella* spp.<br>(*n* = 32)*<br>*Listeria* spp.<br>*Yersinia enterocolitica*<br>*Staphylococcus aureus* | *Campylobacter* spp.<br>*Listeria* spp. | *Campylobacter* spp.<br>*Listeria* spp. |

* Not all samples were tested for *Salmonella*. For explanation see text.