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The Occurrence of *Staphylococcus aureus* on a Farm with Small-Scale Production of Raw Milk Cheese

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ABSTRACT

In recent years, the small-scale production of raw milk products has increased in Norway, and there is some concern that such foods may pose a risk of staphylococcal food poisoning to consumers. The aim of the study was to evaluate potential sources of contamination of raw milk cheese with *Staphylococcus aureus* on a bovine dairy farm with small-scale production. Samples for bacteriological analyses (n = 144) were collected from the animals, the environment, processing equipments, from humans, and from cheeses at various stages of production. *Staphylococcus aureus* was isolated from 10 of 11 cows, the farmer, equipment, the environment, and the cheese. Seventy-five *Staph. aureus* isolates were genotyped by pulsed-field gel electrophoresis, tested for enterotoxin (SE) production by reversed passive latex agglutination, for SE genes by multiplex polymerase chain reaction, and for penicillin resistance by the cloverleaf method. Five different pulsotypes were identified and SE gene fragments were identified in 11 isolates, but no isolates produced SE or were penicillin resistant. *Staphylococcus aureus* was found throughout the farm, and appeared to be spread with the milk to the environment, equipment, and to products. One pulsotype dominated and was identified from most sample sites on the farm. Raw milk products are vulnerable to contamination with *Staph. aureus.* Strategies to reduce the occurrence of *Staph. aureus* in bulk milk are of particular importance on farms where milk is used for raw milk products.
(**Key words:** *Staphylococcus aureus,* raw milk cheese, small-scale production, pulsed-field gel electrophoresis)

Abbreviation key: BA = blood agar with washed bovine erythrocytes, **BP+RPF** = Baird-Parker agar with rabbit plasma fibrinogen, **PFGE** = pulsed-field gel electrophoresis, **PT** = pulsotype, **SE** = staphylococcal enterotoxin.

INTRODUCTION

In recent years, Norwegian agricultural politics has encouraged the production of niche foods including small-scale dairy products. A number of small-scale dairies have been started, and many use raw milk for production. The increasing production of raw milk products in Norway has brought forward certain food safety concerns.

Staphylococcus aureus was recently detected in 75% of 220 samples of bovine bulk milk (Jørgensen et al., 2005). This is of concern because bacteria in raw milk may contaminate raw milk products and create a risk of food poisoning to consumers (Zottola and Smith, 1993; Headrick et al., 1998). Prevalences of 20 to 38% of *Staph. aureus* in Norwegian raw milk products have been reported (Kruse, 1999, 2000; Haugmo, 2001; Jørgensen et al., 2005), and in a Swedish study, coagulase-positive staphylococci were detected in 38% of on-farm manufactured raw goat cheeses (Tham et al., 1990). In France, *Staph. aureus* is reported to be the most frequent cause of foodborne disease from raw milk cheeses (De Buyser et al., 2001), and in Norway, outbreaks of *Staph. aureus* food poisoning have been attributed to raw goat cheese (Aas et al., 1992; Schønberg and Wåltorp, 2001), raw cow cheese (Berg et al., 1996), and potato-mash made with raw milk (Loncarevic and Mathisen, 2004).

Staphylococcus aureus food poisoning is caused by ingestion of food containing preformed enterotoxins (**SE**). Symptoms have a rapid onset and may include nausea, vomiting, and diarrhea (Jablonski and Bohach, 1997). Eighteen different SE have been described and designated SEA–SEE, SEG–SER, and SEU (Dinges et al., 2000; Fitzgerald et al., 2001; Jarraud et al., 2001; Kuroda et al., 2001; Orwin et al., 2001, 2002; Letertre et al., 2003; Omoe et al., 2003). In favorable conditions, *Staph. aureus* may grow and produce SE in foods, and because the SE are stable with respect to heat and

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storage they may be present in foods where viable *Staph. aureus* are absent (Jablonski and Bohach, 1997).

Dairy animals are probably the main source of contamination of raw milk with *Staph. aureus* (Bone et al., 1989; Gilmour and Harvey, 1990; Asperger, 1994; Vautor et al., 2003). In particular, dairy animals with subclinical *Staph. aureus* mastitis may shed large numbers of *Staph. aureus* into the milk. However, contamination of raw milk and raw milk products from human handling or from the environment during manufacture is also possible.

Environmental conditions such as temperature, pH, water activity, salt concentration, and competing microflora influence *Staph. aureus* growth and SE production (Genigeorgis, 1989), and various milk production techniques may be used to prevent the growth of pathogenic bacteria in the products. Nevertheless, it is important that contamination of milk and milk products with *Staph. aureus* is minimized, and a further understanding of the spread of *Staph. aureus* from dairy animals, humans, and the farm environment to milk and raw milk products is needed.

The aim of the present study was to evaluate potential sources of contamination of raw milk cheese with *Staph. aureus* in a typical Norwegian dairy farm with small-scale production.

MATERIALS AND METHODS

The Farm

The study was performed in September 2003 in the summer-farm facility of a Norwegian dairy farm with 11 lactating cows of the Norwegian Red breed. From June through September, the animals pasture at the summer farm where raw milk products are manufactured in a small dairy facility. The facilities are licensed for production and local retail of raw milk products by the food hygiene authorities.

During summer, machine milking is performed in a small cowshed. The milk-room has wash facilities for cleanup and sanitation of milking equipment, and a bulk tank for cooling and storage of milk. Bulk milk is either delivered to a cooperative dairy or used for manufacture of raw milk products on the premises. The dairy consists of a small production room and a room for cheese maturation. Production techniques are largely manual, and the produce includes soured cream and a semihard cheese.

Sampling

Sampling was performed on 2 consecutive days. On d 1, samples were collected from the cowshed and milk room, and on d 2, from the dairy during cheese-making. Samples were also collected from cheese at different stages of maturation.

Swab samples were collected with sterile cotton swabs (Celsis-Lumac, Newmarket, UK) that were moistened in sterile peptone water, rolled on the test surface, and placed in 4 mL of Voegel-Johnson broth (Oxoid, Basingstoke, UK) with 0.5% agar in sterile glass test tubes. Sterile cotton plugs were used to sample floor drains, test material was absorbed, and the plugs were transferred to sterile polypropylene test tubes (Greiner Bio-one, Frickenhausen, Germany). Fluid and solid samples were collected at volumes of 50 to 100 g in sterile plastic tubes.

The udder and teat skin of each cow were swabbed by moving a swab from the udder skin to the tip of the teat. Quarter milk samples were collected from each cow according to Harmon et al. (1990). The nasal membrane of each cow was swabbed 1 to 5 cm inside one nostril and the vaginal mucosa was swabbed in the vestibular area. Each animal was inspected for teat wounds and wounds or excoriations in other locations, which were swabbed if observed.

From the cowshed and milk-room pre- (n = 8) and postmilking (n = 8) swab samples were collected from milking equipment (teatcup liners, claw pieces of milk machine clusters, milk filter) and the environment (sink in milk room, tap in milk room, door handles, hosepipe, washing brush). In addition, 2 premilking samples were collected from washing water for udders and teats and from tap water, and a postmilking environmental sample was collected from the milk-room floor drain. The farmer's nose was swab-sampled premilking, and his hands were swab-sampled pre- and postmilking. On d 1, a bulk milk sample was collected during transfer of milk from the farm tank to the milk truck.

On d 2, 80 L of the morning milk was used to produce a semihard cheese. Swab samples were collected pre- (n = 16) and postproduction (n = 10) from production equipment (cheese molds, vat, plastic scoop, curd cutter, palette knife, strainer, cheese knife, wooden spoon, cheesecloth, cutting board), the environment (wooden shelf in storage room, tap, sink, door handle, window sill, floor in storage room, wash brush). In addition, an environmental sample was collected postproduction from the dairy floor drain. The cheesemaker's nose was swab-sampled preproduction, and her hands were swab-sampled pre- and postproduction.

Cheese making was started approximately 1 h after milking. The cheese-maker wore clean latex gloves during cheese production. Single samples were collected from the cheese at various stages of production. Milk for cheese making (bulk milk from d 2) was sampled from the vat. Mesophilic and thermophilic starter cultures were sampled. Starters had been collected from