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Outbreak of staphylococcal food poisoning among children and staff at a Swiss boarding school due to soft cheese made from raw milk

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ABSTRACT

On October 1, 2014, children and staff members at a Swiss boarding school consumed Tomme, a soft cheese produced from raw cow milk. Within the following 7 h, all 14 persons who ingested the cheese fell ill, including 10 children and 4 staff members. Symptoms included abdominal pain and violent vomiting, followed by severe diarrhea and fever. We aim to present this food poisoning outbreak and characterize the causative agent. The duration of the incubation period was dependent of the age of the patient: 2.5 h in children under 10 yr of age, 3.5 h in older children and teenagers, and 7 h in adults. The soft cheese exhibited low levels of staphylococcal enterotoxin (SE) A (>6 ng of SEA/g of cheese) and high levels of staphylococcal enterotoxin D (>200 ng of SED/g of cheese). Counts of 10^7 cfu of coagulase-positive staphylococci per gram of cheese were detected, with 3 different *Staphylococcus aureus* strains being present at levels $>10^6$ cfu/g. The 3 strains were characterized using *spa* typing and a DNA microarray. An enterotoxin-producing strain exhibiting *sea* and *sed* was identified as the source of the outbreak. The strain was assigned to *spa* type t711 and clonal complex 8, and it exhibited genetic criteria consistent with the characteristics of a genotype B strain. This genotype comprises bovine *Staph. aureus* strains exclusively associated with very high within-herd prevalence of mastitis and has been described as a major contaminant in Swiss raw milk cheese. It is therefore highly likely that the raw milk used for Tomme production was heavily contaminated with *Staph. aureus* and that levels further increased due to growth of the organism and physical concentration effects during the cheese-making process. Only a few staphylococcal food poisoning outbreaks involving raw milk products have been described. Still, in view of this outbreak and the possible occurrence of other foodborne pathogens in bovine milk, consumption of raw milk and soft cheese produced from raw milk constitutes a health risk, particularly when young children or other members of sensitive populations are involved.
Key words: outbreak investigation, *Staphylococcus aureus*, raw milk cheese, genotype B

INTRODUCTION

Staphylococcal food poisoning (**SFP**) is one of the most prevalent causes of foodborne intoxication worldwide, resulting in an estimated 241,148 cases and 6 deaths in 2006 in the United States alone (Scallan et al., 2011). After a short incubation period of 2 to 6 h, patients exhibit nausea, followed by violent vomiting and diarrhea (Tranter, 1990). As clinical symptoms are typically self-limiting, it is estimated that only 10% of SFP patients visit a hospital (Holmberg and Blake, 1984). Consequences include pronounced underreporting of the disease and scarce scientific data on the characteristics of *Staph. aureus* strains causing SFP.

Staphylococcal food poisoning is caused by consumption of staphylococcal enterotoxins (**SE**) formed by *Staph. aureus* in food. To date, more than 20 different SE and SE-like superantigens have been described (Hennekinne et al., 2012), but only a few have been demonstrated to elicit an emetic response in a monkey feeding assay. Staphylococcal enterotoxins that have been shown to exhibit emetic activity include the classical enterotoxins SEA, SEB, SEC, SED, and SEE, and, to a limited degree, some newly described enterotoxins (Thomas et al., 2007).

In SFP outbreak investigations, identification of the causative strain can be challenging, as SE are highly heat resistant. Even when the organism is inactivated and can no longer be isolated from a food item, the highly stable enterotoxins formed by *Staph. aureus* can still cause SFP (Le Loir et al., 2003). In addition, identification of the causative strain in an outbreak investigation is aggravated by the high prevalence of

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Staph. aureus in humans and animals. *Staphylococcus aureus* persistently colonizes the anterior nares of 20 to 30% of the human population (van Belkum et al., 2009), causes a multitude of infections in humans and livestock, and can be isolated from a wide range of food items (Baumgartner et al., 2014). The organism also represents a common cause of bovine mastitis and can be detected in bulk tank milk at prevalence rates of 27 to 42% (Oliver et al., 2009).

On October 1, 2014, children and staff members at a Swiss boarding school consumed Tomme, a soft cheese produced from raw milk. Within 7 h, all 14 persons who had consumed the cheese fell ill, among them 10 children and 4 members of the staff. Based on the short incubation time, as well as the clinical symptoms, SFP due to consumption of the raw milk cheese was considered a possible cause of the outbreak.

MATERIALS AND METHODS

Enumeration and Isolation of Coagulase-Positive Staphylococci, Cell Lysis, and DNA Extraction

Coagulase-positive staphylococci (**CPS**) present in the Tomme soft cheese (that had been stored at 4°C) were enumerated according to EN ISO 6888-2 (ISO, 1999).

Different morphologies of colonies forming an opaque fibrin halo on rabbit plasma fibrinogen agar (Oxoid, Basel, Switzerland) after 48 h of incubation at 37°C were subcultured on 5% sheep blood agar (Oxoid) and incubated overnight at 37°C. DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) and following the manufacturer's instructions.

spa Typing

The polymorphic X region of the *spa* gene was determined as previously described (Wattinger et al., 2012). Briefly, *spa* was amplified using spa-1113f (5′-TAA AGA CGA TCC TTC GGT GAG C-3′) and spa-1514r primers (5′-CAG CAG TAG TGC CGT TTG CTT-3′) (Aires-de-Sousa et al., 2006) and the GoTaq PCR system (Promega AG, Dübendorf, Switzerland) at the following reaction conditions: (1) 5 min at 94°C; (2) 35 cycles of 45 s at 94°C, 45 s at 60°C, and 90s at 72°C; and (3) 10 min at 72°C. The PCR amplicons were purified using the MinElute PCR Purification Kit (Qiagen). Sequencing was outsourced (Microsynth, Balgach, Switzerland) and *spa* types were determined using the *spa*-server (http://spa.ridom.de/; Harmsen et al., 2003).

Microarray-Based Genotyping

The *Staph. aureus* Genotyping Kit 2.0 (Alere Technologies GmbH, Jena, Germany) was used to further characterize the *Staph. aureus* strains. This analytical tool can be used to predict clonal complex (**CC**) assignment (Monecke et al., 2008) and to determine the presence or absence of over 300 resistance and virulence genes, including genes encoding the major SE (*sea*–*see*), newly described SE (*seg*, *seh*, *sei*, *sej*, *sek*, *sel*, *seq*, *ser*), and enterotoxin-like superantigens (*selm*, *seln*, *selo*, *selu*).

Detection of SE in Cheese

To screen for all major SE, an extract of the cheese sample was prepared and analyzed using SET2 miniVIDAS (bioMérieux, Lyon, France) according to the manufacturer's recommendations. The SET-RPLA (staphylococcal enterotoxin–reversed passive latex agglutination) kit (Oxoid) was subsequently used to enable semiquantitative detection of SEA, SEB, SEC, and SED.

RESULTS AND DISCUSSION

All persons who consumed the raw milk cheese fell ill, among them 10 children and 4 members of the staff (Table 1). Although the average incubation time was 4.4 h, the individual duration of the incubation period depended on the age of the patient. Only 2.5 h after consumption of the cheese, the 2 youngest children (age 8 and 9 yr) complained about abdominal pain, fever, and aching limbs that progressed quickly to emesis, followed by severe diarrhea, and fever. One hour later, the older children (10–16 yr) exhibited the same symptoms, followed 3.5 h later by the adults (31–57 yr). One person sought medical care and was treated.

We detected 10^7 cfu presumptive CPS per gram of cheese in the Tomme sample. Different morphologies of coagulase-positive colonies exhibiting a phenotype consistent with *Staph. aureus* were visible on RPF agar (Figure 1), indicating contamination of the product with more than one *Staph. aureus* strain. Using SET2 miniVIDAS to screen for major SE, the cheese tested positive for SEA, SEB, SEC, SED, and SEE in 25 g of product. Subsequently, the SET-RPLA kit was used for semiquantitative detection of SEA, SEB, SEC, and SED, identifying low levels of SEA (>6 ng of SEA/g of cheese) and high levels of SED (>200 ng of SED/g of cheese) in the Tomme soft cheese. Staphylococcal enterotoxin A is the most common SE recovered from food-poisoning outbreaks (78%) and is reported to cause symptoms of intoxication in humans at a total dose of

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