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Fluctuation in contamination dynamics of *L. monocytogenes* in quargel (acid curd cheese) lots recalled during the multinational listeriosis outbreak 2009/2010

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ABSTRACT

For the first time it has been possible to determine the contamination level of *Listeria monocytogenes* in the very cheese lots of acid curd cheese that caused a multinational outbreak between 2009/2010. The listeriosis outbreak accounted for 34 clinical cases and eight deaths. The cheese, which was distributed in Austria, Germany, the Czech Republic, Poland and Slovakia, was recalled on the 23rd January 2010. All recalled lots were immediately investigated after call back from the retail market. The company manufactured two different cheese types, (i) red smear ripened – and (ii) mold coated/white veined – acid curd cheese. Depending on the lot production dates, cheese samples (n = 1045) were analyzed at three different time points: (i) beginning to mid shelf-life (lot nos. 15–18; production period 5.1.2010–13.1.2010); (ii) end of shelf-life (lot nos. 9–18; production period 21.12.2009–13.1.2010) and, (iii) ≤46 days after the expiry date (lot nos. 1–18; production period 1.12.2009–13.1.2010). Qualitative and quantitative examinations of cheese samples were performed according to ISO 11290-1&2. Examination of the samples, according to ISO 11290-1, resulted in 16 *L. monocytogenes* positive (red smear type) and two negative lots (mold coated type). These results were confirmed by a combined enrichment/real-time PCR method. The contamination values obtained by quantitative ISO 11290-2 varied from ≤*log* 2 cell forming units (CFU)/g to *log* 8.1 CFU/g. Three out of sixteen *L. monocytogenes* positive lots revealed a contamination level of ≤*log* 2 CFU/g at the beginning of their shelf-life when stored at 4 °C. Nevertheless, by increasing the storage life and/or the storage temperature (15, 22 °C) the contamination level could be raised to between *log* 5 and *log* 6 CFU/g. Our data indicate that 81.3% (13/16) of the recalled red smear quargel cheese lots were highly contaminated with *L. monocytogenes*. All this implies that the main contamination of the quargel cheese took place during the red smear process and that quargel cheese can easily support growth of *L. monocytogenes*.

1. Introduction

Considerable work has been done to define the behavior of *L. monocytogenes* during manufacture and ripening of different types of cheese. Several surveys have indicated that soft cheeses ripened with mold or bacteria are most frequently contaminated with *L. monocytogenes* (Terplan et al., 1986; Rudolf and Scherer, 2001), while the contamination is localized almost exclusively on the cheese surface, the so-called rind (Farber and Peterkin, 1991). According to Rudolf and Scherer (2001) the foodborne pathogen appears frequently on red smear cheeses, even when pasteurized milk has been used for cheese making. It has been shown that the frequent contamination of these types of cheeses results from a post-process contamination arising from traditional methods of cheese production, such as "old-young-smearing".

First proof that milk products could be responsible for listeriosis outbreaks was provided by Fleming et al. (1985). Over the past 27 years *Listeria monocytogenes* has become increasingly important as a food-associated pathogen, and dairy products are seen as the main sources of contamination (Oliver et al., 2005). From June 2009 until February 2010, an outbreak of invasive listeriosis affected 25 persons in Austria, eight in Germany and one in the Czech Republic. Eight of the 34 cases in this outbreak ended fatally. An epidemiological investigation revealed quargel cheese produced by an Austrian manufacturer to be the source of infection (Fretz et al., 2010).

Classic quargel is an Austrian specialty sour milk cheese with a red smear (Mair-Waldburg, 1974). The starting raw material for quargel cheese is curd cheese made from skimmed pasteurized milk. 0.5% to 1.0% ripening salts, and between 2 and 3% common salt and starter cultures are added to the curd cheese. This mixture is ground in a mill to

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yield a homogeneous mixture and subsequently rolled and shaped into typical round cheese masses or cakes from 125 to 200 g. These small cakes of cheese are then spread onto boards and transferred to a sweat room, where the cheese matures for a further 24 h (±2) at a temperature of between 26 and 30 °C and a relative air humidity of 95%. Following the sweating procedure the cheese cakes are coated with the ripening culture. If a mold cheese is required, they are sprayed with a fungal culture such as *Penicillium* spp. Red smear cheeses typically involve use of a culture of *Brevibacterium linens*. Consequential ripening of the coating at 16 °C takes place over a further two days, where maturation takes place from the outside of the cheese to the inside. Following a cooling phase of two days at 12 °C, the cheeses can then be packaged in gas permeable plastic wraps. After packaging the cheese is stored at 4 to 6 °C. Three days after packaging the cheese is about a quarter ripe and can be dispatched. A consumable cheese is one that has ripened for between six and up to 13 days, that is, by at least one-third. Alternatively, many consumers prefer the whole cheese to undergo proteolytic maturation, which takes up to 22 days (Spreer, 1995). According to the company's product specification, the shelf life of quargel cheese was 50 days and was calculated from the production date, which was the homogenization step of the curd cheese.

On the 23rd January 2010 quargel cheese lots were withdrawn from the Austrian, German, Slovakian and Czech markets. According to the official press release of the company, 50 to 60 t were recalled, stating that three out of five cheese samples tested positive for *Listeria*. However, at that time the company press release explicitly pointed out that the limit value of 100 colony forming units (CFU) per gram of cheese had not been exceeded, the cheeses remained consumable, the recall was voluntary and not officially requested (Schiffl, 2010).

On the 27th January the Institute of Milk Hygiene, Milk Technology and Food Science in Vienna was asked for outbreak clarification and reorganization of the company. That same day it was possible to inspect the company and to seize all recalled lots.

The aim of this study was: (i) to determine the contamination level of all recalled lots and, (ii) to conduct durability studies at 4, 15 and 22 °C in order to simulate proper and improper storage conditions.

2. Material and methods

2.1. Recalled cheese lots

A cheese lot, which comprised approximately 3000 cheese cakes, was defined as one day's production, which was one filling of the homogenizer, respectively. Between 2009 and 2010 the Austrian company Prolactal manufactured two different cheese types: (i) red smear ripened (*Brevibacterium linens;* BLO, Cargill France SAS, La Ferte sous Jouarre, France) and (ii) mold coated/white veined-(*P. candidum*; PC TAM5, Cargill France SAS, La Ferte sous Jouarre, France) acid curd cheese. Both types of cheese were marketed as trademarks and under the company's brand name in different cake sizes of 125 g, 150 g or 200 g. Nevertheless mold coated quargel had a smaller market size, and was exclusively manufactured for the German market (mold coated cheeses comprised 11% of production). Approximately 16 t of quargel cheese was produced per week. Almost 50% of the cheese was exported to the German market and small amounts to the Czech Republic and Slovakia.

Following recall on the 23rd January 2010 more than a hundred kilograms of cheese (6 kg cheeses per lot) were sent to the Institute of Milk Hygiene, Milk Technology and Food Science, Vienna. On receipt the cheese lots were stored at 4 °C prior to analysis.

2.2. Cheese sample preparation

Depending on the weight of the cheese cakes (125, 150 or 200 g) twenty-four, twenty or fifteen pieces were randomly selected to make a 600 g pooled sample of each cheese lot (Fig. 1, Section 1). The 600 g pooled samples were obtained by cutting wedge shaped portions from each of the 15, 20, or 24 cheese lots using an ethanol-flamed stainless steel knife. The first cuts divided the cheeses into five parts (each 25, 30 or 40 g), depending on the pack size of the cheese (125, 150 and 200 g portions). The knife was flamed with ethanol before each operation. Pooled samples (A_x–E_x) were placed into sterile stomacher bags and homogenized in a sterile blender (Fig. 1, Section 2).

All durability studies were performed in triplicate. Briefly, 25 g and 10 g, respectively, were aseptically transferred from the pooled sample into a stomacher bag and stored at 4 °C, 15 °C or 22 °C, depending on each particular experiment (Fig. 1, Section 3).

2.3. Isolation, quantification and confirmation of L. monocytogenes

Qualitative examination of the samples was performed according to the standard method ISO 11290-1 (Anonymous, 1996) (Fig. 1, Section 4).

Briefly, 25 g samples were added to 225 mL Half-Fraser (HF) broth (Merck, Darmstadt, Germany), homogenized for 3 min in a stomacher (Lab Blender 400 Seward, UK) and incubated for 24 h at 30 °C. Subsequently 0.1 mL of the HF broth was transferred to tubes containing 10 mL of Full-Fraser Broth (FF, Merck) and incubated at 37 °C for 48 h. Both enrichment broths (HF and FF) were streaked onto duplicate plates of Palcam and OCLA agar (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom). The plates were incubated for 48 h at 37 °C and observed for the presence of typical *Listeria* spp. colonies. Additionally, quantitative examination was carried out, according to ISO 11290-2 (Anonymous, 1998). Populations of Listeria were enumerated on Palcam and OCLA agar (Oxoid) by performing serial dilutions on all samples. To achieve a lowest detection limit of 1.0 log CFU/g cheese, additionally, 1 mL of initial suspension was plated in quadruplicate onto Palcam and OCLA agar (Anonymous, 1998).

In contrast to conventional microbiology, where only five colonies are selected from a colonized plate for *Listeria* species confirmation, in this study the whole agar surfaces (including all presumptive *Listeria* spp. colonies) of incubated Palcam and OCLA plates were harvested by swabbing. Bacterial suspensions were subjected to PCR after a short Chelex-based DNA isolation method as described by Rossmanith et al. (2006). Two PCR reactions were performed: one targeting the 16S rRNA gene specific for all *Listeria* spp. and the *hly* gene specific for *L. monocytogenes* (Border et al., 1990), and the other targeting the *iap* gene of *Listeria* spp., yielding fragments typical of each species or group of species (Bubert et al., 1999).

The combined enrichment/real-time PCR method was performed, according to Rossmanith et al. (2006). Twenty-five grams of the A_x sample set of quargel cheese were added to 225 mL HF-medium, according to ISO 11290-1 (Fig. 1, Section 4). After 24 h incubation at 30 °C a nine mL aliquot was removed and centrifuged at 50 *g* for 2 min. The pellet was discarded and the supernatant centrifuged at 3220 *g* for 10 min. The resulting pellet was subjected to bacterial target DNA extraction using the NucleoSpin® tissue kit (Macherey — Nagel, Düren, Germany) and the support protocol for Gram-positive bacteria, according to the manufacturer's instructions.

Real-time PCR detection of *L. monocytogenes* by targeting a 274 bp fragment of the *prfA* gene was performed, according to the previously published format (Rossmanith et al., 2006). Conventional and real-time PCR reactions were performed in an Mx3000p real-time PCR thermocycler (Stratagene, La Jolla, CA, USA). The 25 μL volume contained 5 μL of DNA template. Real-time PCR results were expressed as bacterial cell equivalents (BCE). The copy number of the *prfA* gene was determined by assuming, based on the molecular weight of the genome of *L. monocytogenes*, 1 ng of DNA equaled 3.1×10^5 copies of the entire genome, and that the *prfA* gene is a single copy gene (Nelson et al., 2004; Rossmanith et al., 2006). All PCR reactions were performed in duplicate. PCR products of conventional PCR were separated in 1.5%