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The impact of shelf life on exposure as revealed from quality control data associated with the quargel outbreak

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ABSTRACT

A cluster of 34 human cases of listeriosis was traced to consumption of contaminated quargel cheese, a sour milk specialty sold in Austria, Germany and Czech Republic. Here, we try to assess how many portions were consumed by the Austrian population at a certain contamination level (CL). In total, 1623 cheese lots were produced during the outbreak period resulting in > 3 million portions of cheese delivered to the market. From 650 sets of quality control data provided by the food business operator, we reconstructed the contamination scenario over time and identified 84 lots that were found to be positive. With regard to another sixteen lots, a CL was found ranging from one to 3,84 $\log_{10}$ CFU *L. monocytogenes*/g, measured in product stored between one to 23 days after production. However the number of storage days at home before consumption is unknown. To resolve this issue, we modelled the theoretical CL of the product if consumed either 20, 30, 40 or 50 days post production. We found that 10 lots (approx. 27,350 portions) would have been contaminated at CLs higher than 3 $\log_{10}$ CFU *L. monocytogenes*/g if all cheese had been consumed after 20 days of storage. This number shifts to 20 lots (approx. 54,700 portions) after 30 days of storage. If all cheese had been consumed at the end of shelf life (50 days of storage), theoretically 242,5 lots would have exceeded a CL of 6 $\log_{10}$ CFU *L. monocytogenes*/g. We concluded that the extended shelf life given to the product was a driver of the outbreak scenario. It is stunning to note that so few cases were reported in spite of consumers' massive exposure to *L. monocytogenes*. We hypothesized that a low pathogenicity of both quargel outbreak clones (QOC1 and QOC2) could have contributed to this discrepancy. Our hypothesis was falsified since both strains QOC1 and QOC2 are fully virulent in an oral infection mouse model, showing even higher pathogenicity than the reference strain EGDe.

1. Introduction

Listeria monocytogenes is a Gram-positive facultative intracellular pathogen responsible for listeriosis, a rare but potentially severe infection in humans and animals (Vazquez-Boland et al., 2001). The infection is transmitted to recipients via consumption of contaminated food and feed. Outbreaks of listeriosis are reported annually worldwide and recent episodes included such different food commodities as meat products, hard cheese and cantaloupe (Eurosurveillance editorial, 2015). This indicates that *L. monocytogenes* is an organism (re)contaminating and surviving many different food chains (Allerberger and Wagner, 2010; NicAogain and O'Byrne, 2016). In spite of being distributed widely in food processing environments (Ferreira et al., 2014; Larsen et al., 2014; Muhterem-Uyar et al., 2015), sources responsible for outbreaks are often difficult to trace back and the dynamics of the contamination pattern remain unknown. In the outbreak of invasive listeriosis affecting 34 persons in Austria, Germany and the Czech Republic, an exceptionally large amount of information regarding the source and the dynamics of contamination could be collected (Fretz et al., 2010a; Fretz et al., 2010b). Eight out of the 34 outbreak cases reported were with fatal outcome. Almost all of the clinical cases occurred in elderly males suffering from underlying disease (Fretz et al.,

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2010b). That male consumers were almost exclusively affected is explainable by the outbreak source: a soft cheese that matures even after packaging resulting in an intense taste and odor after extended periods of storage. Previous research revealed that the initially dominating outbreak-associated clone (QOC1) was reduced in prevalence and a second clone (QOC2) became the major contaminant before production stop and recall was initiated (Schoder et al., 2014). In the meanwhile a body of information has been collected including growth parameters in the particular product (Schoder et al., 2012), reliability of the quality control system in place (Schoder et al., 2013), distribution of molecular types in cheese lots at the time point of recall (Schoder et al., 2014), genome sequence analysis and the in vitro virulence potential of the incriminated outbreak clones (Rychli et al., 2014). Here, we add information by achieving, from a set of 650 quality control data spanning the whole duration of the outbreak episode, an estimate of contaminated lots and portions that were delivered to the market. We found three sources of information hampering analysis: (i) distribution of *L. monocytogenes* within cheese lots, (ii) timespan for storage at home before consumption and (iii) a realistic assumption of the pathogenicity of both outbreak clones. The distribution issue was tackled by a re-evaluation of already existing data that were received from recalled lots. To solve the shelf-life issue, we modelled the impact of storage at home by using predictive microbiology. Additionally we analyzed the pathogenicity of both outbreak strains and the reference strain EGDe in an oral infection mouse model.

2. Material and methods

2.1. Patient's histories

A case report was included in this study if it was unequivocally proven that the patient was infected by *L. monocytogenes:* by culturing *L. monocytogenes* in a clinical specimen and if the PFGE type of the clinical isolate matched one of the two *L. monocytogenes* PFGE types collected during the outbreak. Out of 29 Austrian cases, three cases did not fulfil this definition. Conclusively, 26 patients' records were included in this study. The data of this study exclude data from five non-Austrian patients that were also reported to be affected by the outbreak (Fretz et al., 2010a; Fretz et al., 2010b). Regarding the vulnerability for an infection of listeriosis, we divided the patients into three categories: (i) patients otherwise clinically healthy or with indication of only minor health deficiencies (such as elevated blood pressure), (ii) patients with records reporting massive health deficiencies but with no indication of tumorous malignancies (such as renal insufficiency, diabetes, cirrhosis) and (iii) cancer patients.

2.2. Food chain data

A detailed description of the cheese making process is given by Schoder et al. (2012). Briefly, the cheese was manufactured from skim milk curd that was bought and stored at 4 °C at the food business operation (FBO) unopened for up to one week. Curd was tested repeatedly for the presence of *L. monocytogenes* and was always negative for contamination with *L. monocytogenes* (data not shown). The curd was then crushed, milled and a starter culture and salt were added. The curd got formed, placed on draining racks and transferred to a “sweating room”. The shelves were turned several times to permit efficient dripping of whey. Following the “sweating” procedure the cheese cakes were sprayed with ripening culture by using a spraying lance; two types of cheese were made: red-smear cheese and mould-coated cheese. Ripening took place in ripening cellars at 16 °C for two days. Cheese was then wrapped in foil and put on stock. After storage time of 10 to 14 days the cheese was delivered to an intermediary and from there retailed via supermarkets.

2.3. Data assessed from the quality control system in place at the plant

It is to emphasize that none of the authors have contributed to investigations or given consultancy to the food business operation during the outbreak episode. The Institute of Milk Hygiene was called to work on a follow-up of the outbreak after the production was ceased in January 2010. The case is closed at court and the sentences have been pronounced. Despite of a retrospective nature, this data are of value since they assess exposure during a real outbreak case. We calculated the timespan of the outbreak from 19th of May 2009 (first report of a case infected with outbreak clone 1 (QOC1) to the 25th of January 2010. On January 25th, 2010, the delivery of lots on stock was stopped after intervention by the public health authorities and 18 mostly highly contaminated food lots were recalled from the market thereafter (Schoder et al., 2014). White mould cheese lots were always negative what led us to conclude that the contamination occurred during the smearing step. The outbreak terminated in February 2010 when the last four cases with infections caused either by QOC1 and QOC2 were reported by the public health authorities. During the mentioned timespan, 1623 batches of cheese were produced. A batch encompassed approximately 450 kg of cheese and was packaged either in 150 g (approximately 70% of all lots) or 250 g (approximately 30%) portions. Based on these data a lot encompassed approximately 2205 portions of 150 g (3 cakes) and 530 portions of 250 g portions (five cakes) of cheese. We assumed that both the 150 g and the 250 g cheese portions are consumed per meal. Therefore we calculated that around 2735 cheese portions were marketed per lot. Encompassing all lots, 730,350 kg of cheese were produced during the outbreak period what resulted in > 4.4 million portions. Since approximately 30% of the cheese products were exported, we concluded a domestic share of quargel cheese totalling to 511,245 kg. Therefore the total number of portions retailed in Austria was estimated at 3,107,000 meals. The number of contaminated lots was revealed by carefully assessing the records of the quality control system of the FBO. Nevertheless the numbers are approximations as some of the values are based on justified estimations (e.g. the ratio between 150 g and 250 g packages; the export market share). Since not all lots were tested we extrapolated the data as revealed from the 650 available data points to the total of 1623 lots of cheese produced (multiplication factor 2.5). All data sets were produced by laboratories contracted by the enterprise and used the standard analytical tools as laid down in ISO 11290:1 and 11,290:2 (Anonymous, 1996, 1998).

2.4. Growth model for predictive microbiology

Growth of *L. monocytogenes* in the packaged products placed on sale for the different storage durations was modelled as described in Schoder et al. (2013) in order to estimate the likely different final levels of *L. monocytogenes* at consumption. Briefly, the logistic equation with decay (Rosso et al., 1996) was used for simulation of *L. monocytogenes* growth under the aforementioned temperature and dynamic pH conditions, assuming instantaneous adaptation of μ_{max} at the momentary intrinsic and extrinsic conditions of cheese during storage.

$$X_{(t)} = \begin{cases} X_0, & t \le lag \\ \frac{x_{max}}{1 + (x_{max} - 1) \cdot \exp[-\mu_{max}] \cdot (t - lag)} & t > lag \end{cases} \quad (1)$$

The values of $\boldsymbol{\mu_{max}}$ for a given temperature, pH and a_w of cheese along the aforementioned chain scenarios were predicted by a gamma concept-based model (Augustin et al., 2005) with interaction terms (Le Marc et al., 2002; Mejlholm et al., 2010), as described in the following equation:

$$\mu_{max} = \mu_{opt} \cdot CM_2(T) \cdot CM_1(pH) \cdot SR(a_w) \cdot \xi \quad (2)$$

where $CM_2(T)$, $CM_1(pH)$ and $SR(a_w)$ are the gamma-terms for the respective effects of temperature, pH and a_w, and ξ the interaction term