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Salmonella in table eggs from farm to retail – When is cooling required?

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ABSTRACT

Storage time and temperature are known to be important factors in determining *Salmonella* growth in table eggs. It is assumed that growth of *Salmonella* within eggs starts when the egg yolk membrane breaks down, allowing *Salmonella* to enter and grow. The length of time for which the yolk membrane remains intact is time and temperature dependent.

The present study addresses the question whether the current legal requirement in Germany to cool table eggs marketed 18 days after laying needs to be updated. Detailed data on the current storage and transport conditions in Germany were collected. On the basis of these data, typical combinations of temperature and time were selected. For these different time-temperature scenarios, the consequences regarding the probability of growth of *Salmonella* Enteritidis inside the eggs were calculated. A probabilistic model consisting of nine modules reflecting storage/transport stages from farm to retail in Germany was used to calculate whether the yolk membrane remains intact during the individual steps. Growth of *Salmonella* was simulated without cooling (room temperature) as well as with two different cooling scenarios (4–6 °C and 8–12 °C), which are temperature ranges used in Germany by various retailers. Simulations of these scenarios resulted in relative low numbers of eggs with *Salmonella* growth until purchase (89 out of 50,000 eggs in the uncooled scenario, 10 out of 50,000 eggs stored at 8–12 °C and 5 out of 50,000 eggs stored at 4–6 °C). These results show that for an average egg trading time of 7.5 days, as was observed for Germany, the probability for the consumer to purchase eggs where no *Salmonella* growth has started and yolk membrane integrity still exists is high. However, the model supports the necessity for egg cooling after the yolk membrane integrity time is exhausted. Furthermore, the model shows clearly that cooling will extend the time period before the egg yolk membrane breaks down. According to our results, the requirement to cool eggs from the 18th day onwards after laying, as required by legislation in Germany, is reasonable, as it matches the time point of the yolk membrane breakdown for the average egg kept at 18–20 °C. Therefore, continuation of this regulation is strongly supported.

1. Introduction

Salmonellosis is one of the most frequent foodborne diseases in humans in Germany. Although the number of cases has been decreasing in recent years (from nearly 200,000 cases in 1992 to below 20,000 cases in 2013); (Robert Koch-Institut: SurvStat@RKI, http://www3.rki.de/SurvStat, 2014), the number of unreported cases is likely still high. Eggs and egg products are considered the most important source for salmonellosis in humans (EFSA, 2005). Europe-wide, the serovar *Salmonella* Enteritidis is most commonly detected in table eggs (EFSA, 2007). Official food surveillance of table eggs in Germany reports *Salmonella* Enteritidis as the only serovar found in eggs in 2010 (Hartung & Käsbohrer, 2012). Furthermore, this serovar was responsible for 45% of cases of human salmonellosis in 2011 in Germany (Robert Koch Institut (RKI), 2012).

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Although control measures have been introduced to reduce egg-borne outbreaks of salmonellosis, continual assessment and refinement of measures is required. Cooling of table eggs during storage is regarded as an effective method to prevent the growth of pathogens like *Salmonella* spp. (EFSA, 2005). According to regulation (EC) No. 853/2004 laying down specific rules for the hygiene of foodstuffs (Regulation (EC), 2004), eggs should be stored and transported at a constant temperature which guarantees the best hygienic conditions. German legislation requires that eggs are cooled at 5–8 °C from the 18th day onwards after laying (Animal food hygiene regulation (Tier-LMHV), 2007). Furthermore, regulation (EC) No. 853/2004 (Regulation (EC), 2004) stipulates that eggs must be purchased within 21 days after laying and regulation (EC) No 589/2008 (Regulation (EC), 2008), that the expiration date of eggs is set at 28 days after laying.

Salmonella are gram-negative, facultative anaerobic bacteria of the family Enterobacteriaceae. They are able to survive for long periods in the environment. *Salmonella* can grow at temperatures in the range of 10–50 °C (optimum 37 °C). Although no growth occurs at refrigerator or freezer temperatures, *Salmonella* are able to persist at these temperatures.

There are two possible pathways for *Salmonella* to contaminate an egg: the interior of the egg can be contaminated before laying during egg formation inside the oviduct of an infected hen (usually the initial *Salmonella* concentration is low) (Bygrave & Gallagher, 1989; Humphrey, Whitehead, Gawler, Henley, & Rowe, 1991; Shivaprasad, Timoney, Morales, Lucio, & Baker, 1990) or, more frequently, the surface of the egg can become contaminated. This contamination pathway occurs during or after the laying-process, e.g., through faeces of infected hens (De Reu et al., 2006; Messens, Grijspeerdt, & Herman, 2005). *Salmonella* are able to penetrate the egg shell and migrate into the interior of the egg (Baker, 1990; Board, 1966; De Reu et al., 2006). If the temperature changes during storage (e.g. interruption of the cooling chain) condensation can occur on the surface of the shell. Moist conditions enhance the survival of *Salmonella* on the shell (Nasim, Afzal, Ashfaque, Akhtar, & Hur, 1982) as well as the penetration rate of *Salmonella* through the shell (Chen, Anantheswaran, & Knabel, 2002).

During transport and storage until purchase, the egg is exposed to different time and temperature conditions. These environmental factors are crucial for *Salmonella* growth within eggs. In 2002, a risk assessment attempting to quantify the human health risk attributable to *Salmonella* (*S.*) Enteritidis in eggs was carried out by WHO/FAO (World Health Organization (WHO), & Food and Agriculture Organization of the United Nations (FAO), 2002). The model developed for the WHO/FAO risk characterization combines FSIS-USDA/FDA and Health Canada models. The predictive microbiology used in this model was based on limited data regarding the growth of *S.* Enteritidis inside eggs. However, the importance of time and temperature conditions in predicting growth of *S.* Enteritidis in eggs in the WHO/FAO model was emphasized. In the model presented in this paper, this particular question was further investigated and discussed in the context of the German situation.

The aim of this study was to develop and describe, on the basis of the already existing models, a further refined quantitative and probabilistic model which simulates in detail all stages of storage and transport during an egg's "life" until purchase. Different temperature and time regimes were studied. Contamination on the shell surface and the penetration process itself were not considered in this paper. Because we were particularly interested in reducing the probability for *Salmonella* growth in eggs from farm to retail, as this entails legal regulation, storage conditions at consumers' households are not included in the model at present. This paper describes the structure of the probabilistic model used, the estimates for the probability that *Salmonella* growth starts for different uncooled and cooled scenarios, and the recommendations regarding egg storage conditions until purchase. Results will guide risk management decisions on how to control *Salmonella* growth from farm to retail.

2. Materials and methods

2.1. General model structure

Based on the WHO/FAO model (World Health Organization (WHO), & Food and Agriculture Organization of the United Nations (FAO), 2002), a probabilistic model was implemented which consists of nine modules representing the relevant steps of egg production, from the laying area on the farm, to the point of sale at retail level (Fig. 1). In the following description, the abbreviations used were adopted from the FSIS-USDA/FDA model for the sake of transparency.

The simplifying assumption is that growth of *Salmonella* within eggs starts when the membrane around the egg yolk breaks down. The length of the period of yolk membrane integrity, the yolk membrane time, is time and temperature dependent. Thus, the model needs to first calculate how much of this time is used at each processing step and when (time point) the membrane around the egg yolk breaks down. As this needs to be considered for each process step (module) separately, fractions of the total time are calculated. The total yolk membrane time used is characterised by the sum of yolk membrane time fractions (SYMTF) which will be <1 when some yolk membrane time (with the value 1) is left and the membrane is intact and >1 when all time has been used and the yolk membrane has broken down.

As input parameter, the *Salmonella* concentration C_{i-1} in the egg after laying or at the end of the previous module is used. Other input parameters of each module or stage i (with $i = 1, \ldots, 9$) are the module duration t_i (in h) and the temperature T_i, during this stage. The primary output from each module is the number of eggs in which growth of *Salmonella* should be assumed based on the modelled status of the yolk membrane. The latter is derived from the sum of yolk membrane time fractions (SYMTF) for the given module.

2.2. Data input

Data used in module 1 and module 2 (time point of egg laying, collection by the farmer, storage time) were obtained by request from the German Egg Producer Association (Zentralverband Eier e.V.). These data describe the general practice in Germany. Temperature data come from a dataset of daily temperatures in Germany recorded between 1991 and 2010 by the Climate Data Center of the German Weather Service. Extreme values were not considered on the assumption that storage rooms do not exceed temperatures below 5 °C and above 25 °C. Detailed information about storage and transport conditions from module 3 until module 9 used in this model was obtained from questionnaires answered by retail managers or quality control staff. For each of these data inputs, distributions were calculated to describe the range of possible values. Details are given in Table 1 and the next section.

2.3. Module duration and temperature

Values of the parameters of duration t_i and temperature T_i were sampled from the respective distributions reflecting the available knowledge about the model parameters (see Table 1). All chosen distributions were fitted on the available time and temperature data using maximum likelihood estimation (MLE) calculated with