International Journal of Food Microbiology 184 (2014) 55–59

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Performance Objectives for *Salmonella* in fresh pork meat intended to be eaten cooked: How to derive them and verify their achievement

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ARTICLE INFO

Available online 27 May 2014

Keywords:
Salmonella
Pork cuts
Sampling
Performance objectives
Risk management

ABSTRACT

In our study we aimed at deriving performance objectives (POs) for *Salmonella* in fresh pork meat intended to be eaten cooked, using loin chop as a model. Moreover, sampling plans to verify the compliance of meat lots to such POs are presented. Ten lots of product, collected in the same slaughterhouse along a one-year period, were tested for *Salmonella* detection under four different storage stages through the product shelf life. The POs were considered as different target values from *Salmonella* prevalence and were calculated at the 50th percentile of prevalence distributions under each stage. Results obtained indicated that values increased between a minimum of 26.10% of positives after final storage at 14 °C to a maximum of 46.70% of positives after storage at retail. The number of samples to be tested in order to detect at least one positive and verify the compliance to the estimated POs ranged between five, for samples after storage at retail, and ten, for samples stored at 14 °C before the expiration date. For risk management purposes, percentiles different from the 50th can be selected in order to derive the POs as well as the number of samples to be tested in order to verify their fulfillment. Thus, the approach presented in this paper offers different options to risk managers for improving the decision-making process.

1. Introduction

Pork is the most frequently consumed meat in the European Union (Devine, 2003). Management of hazards transmitted to humans by consumption of pork is therefore of major health and economic significance. *Salmonella* is one of the main biological hazards in pork products as it is an important zoonotic pathogen of economic and public health significance. In total, 95,548 confirmed human cases of Salmonellosis were reported in 2011 in the EU plus Iceland and Norway (EFSA, 2013) and a statistically significant decreasing trend was observed over the period 2008–2011. It is assumed that the observed reduction in Salmonellosis cases is mainly a result of the successful *Salmonella* control programs in poultry populations.

Many of the national monitoring programs for *Salmonella* in pig meat and products thereof are based on sampling at the slaughterhouse and meat-cutting plants. In 2011, 19 European Member states together with Iceland and Norway reported data on *Salmonella* in fresh pig meat from investigations with 25 or more samples. Overall, a total of 52,868 fresh pig meat units (single or batch) were tested within the EU and 0.7% of them were positive. Most of them corresponded to single carcass samples (81.4% of total units tested) with 0.6% of *Salmonella* positive carcass. Out of the 9858 batches investigated, 0.9% were positive for *Salmonella*. As regards single samples, most of them were carcass swabs and the area swabbed varied from 400 cm^2 to 1400 cm^2. Although it would be expected that Member States (MSs) swabbing larger areas would be more likely to detect *Salmonella*, the highest proportion of positive carcass swabs was observed in an investigation in Germany where 400 cm^2 was sampled (4.0% of positive results). Spain reported testing of meat samples at the slaughterhouse with 7.5% of positive samples (EFSA, 2013).

The microbiological criteria, reported in the Regulation (EC) No 2073/2005 as food safety and hygienic criteria, define the acceptability of a product or a food lot placed on the market, based on the absence/presence or number of microorganisms and/or quantity of their toxins/metabolites, per unit(s) of mass, volume, area or lot. For *Salmonella*, that Regulation, amended by No 1441/2007, states that it must be absent in five sample units of 10 to 25 g of product, including different categories of meat products, placed on the market during the shelf life. However, Regulation (EC) 178/2002 forced to drive food safety under science-based risk analysis (European Community, 2002). To make a risk-based approach of managing food safety operational, Codex formulated risk-based metrics. Two of these metrics are Food Safety Objective (FSO) and Performance Objective (PO) (Codex Alimentarius Commission, 2004). The FSO is the maximum

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http://dx.doi.org/10.1016/j.ijfoodmicro.2014.05.014
0168-1605/

allowable level of a hazard (in terms of concentration and/or frequency) that can be tolerated in the final product at the moment of consumption. Setting an FSO at the moment of consumption is supported by the International Commission on Microbiological Specifications for Foods (ICMSF), as that is the moment when no change in the hazard level can occur anymore and essentially the consumption event is required to have a possible impact on public health (ICMSF, 2002). FSO is proposed to be a metric that gives guidance to food production and preparation of professionals concerning the expected compliance of foods to consumer protection policy with regard to a possibly associated hazard (De Swarte and Donker, 2005). The FSO sets a target for the food chain but does not specify how the target is to be achieved. Hence, the FSO gives flexibility to the food chain to use different operations and processing techniques that suit best their situation, as long as the maximum hazard level specified at consumption is not exceeded. PO is defined as the maximum frequency and/or concentration of a hazard in a food at a specified step in the food chain before consumption that provides or contributes to an FSO. However, unlike FSO that can only be set by governments, POs can also be viewed as targets for food safety managers controlling food supply chain operations (Gorris, 2005) and allow for considerable flexibility in the selection of appropriate control measures for individual steps in the food supply chain. The goal of this study was to suggest an approach to derive POs for *Salmonella* in fresh pork meat intended to be eaten cooked. Moreover, sampling plans to verify their achievement are presented. For the aim of the study loin chops were selected as target product. However, the suggested approach can be extended to other meat products.

2. Materials and methods

2.1. Sampling

Ten different samplings were conducted between December 2010 and November 2011, once a month, except for March and July, at the same pork processing facility in Italy. During each sampling, 16 packs, containing 300 ± 20 g of loin chops and a temperature-logger inside, were vacuum packaged at the processing facility. The loin chops tested during different samplings belonged to different lots, labeled with numbers between 1 and 10. However, those collected during the same sampling time were obtained from the same lot, meaning from animals reared in the same house and slaughtered at the same age.

For each lot, prevalence of *Salmonella* in the packs of loin chops was evaluated under four different stages, throughout the product shelf life (fixed at 7th day). To set different POs a representative distribution chain was considered in this study: within 2 h from packaging in the food industry (S1); after delivery at a retailer site shop and storage within a display cabinet set at 4 °C up to the fourth or fifth day of the product shelf life (S2); after delivery and storage at retail (i.e., S2) and then transport at car temperature for 45 ± 10 min and storage at 6 ± 1 °C, up to the end of the product shelf life (S3); and after delivery and storage at retail (i.e., S2) and then transport at car temperature for 45 ± 10 min and storage at 14 ± 1 °C, up to the end of the product shelf life (S4). The final storage temperatures (i.e., 6 and 14 °C) were selected representative of median (6 °C) and maximum (14 °C) values recorded in household refrigerators (James et al., 2008; Koutsoumanis et al, 2010).

2.2. Detection and confirmation of Salmonella

During each sampling, for each stage, 4 packs and 3 samples of 25 g each were analyzed. The outer wrapper of each pack containing the loin chop portions was disinfected with 70% ethanol. After slitting the package with a flame-sterilized knife, each meat portion was aseptically cut into several pieces. Afterwards, the three sample units of 25 g were randomly selected from different parts of the sample pack. Finally, the temperature logger from each pack was collected and connected to a computer to upload the registered data.

Each 25 g sample was aseptically transferred to a BagFilter (Interscience), diluted 1:10 in Buffered Peptone Water (CM0895B, Oxoid, Milan, Italy) and homogenized by stomaching for 1 min in a Stomacher 400 (Seward, Worthington, UK) at normal speed. Samples were then incubated for 18 ± 2 h at 37 ± 1 °C for a pre-enrichment and the analysis of samples continued following the reference culture method for the detection of *Salmonella* named ISO 6579:2004. From the 18 h pre-enriched BPW, 0.1 ml was transferred into 10 ml of Rappaport Vassilliadis broth (CM 0669, Oxoid) and incubated for 24 ± 3 h at 37 ± 1 °C. Moreover, 1 ml of the BPW was transferred in 10 ml of Muller Kaufmann novobiocin tetrathionate broth (CM 1048, Oxoid) and incubated for 24 ± 3 h at 41.5 ± 1 °C. After incubation, broths were streaked in duplicate onto Xylose Lysine Deoxycholate agar (XLD) (CM 0469, Oxoid) and Brilliant Green Agar (BGA) (CM 0263, Oxoid). Plates were incubated for 24 ± 3 h at 37 ± 1 °C. Five suspected *Salmonella* colonies per sample were sub cultured in Nutrient Agar (BO 0336, Oxoid) and their identity was confirmed biochemically by using API 20E (bioMérieux, Marcy l'Etoile, France) and by qualitative PCR as previously described (Rijpens and Herman, 2002). In each PCR run, *Salmonella enterica* ser. Typhimurium ATCC 14028 and *Listeria monocytogenes* ATCC 13932 were used as positive and negative controls, respectively.

2.3. Data processing

Data of the presence of *Salmonella* spp. in S1–S4 were processed using Microsoft Excel v2010 (Microsoft Corporation). Positive samples obtained for each stage and lot number were recorded. For modeling purposes, the sensitivity and specificity of the analytical technique were not included so that it was assumed that all samples where *Salmonella* was detected by enrichment (i.e., presence/absence) were truly positive. To describe the uncertainty of the number of positive lots within the same storage stage, a Beta distribution was used for prevalence of *Salmonella* in contaminated pork cuts (P) with two parameters ($\alpha = s + 1, \beta = n - s + 1$); being n the total number of samples and s the positive samples. This distribution is widely used as the description of the uncertainty or a random variation of prevalence (Vose, 2008).

2.4. Setting of risk-based metrics (POs) for Salmonella in pork cuts

POs were considered as different target values from Beta distributions considered for *Salmonella* prevalence. The median of the distribution (50th percentile) was chosen to subsequently derive POs referred to two-class attribute sampling plans ($c = 0$). Note that pork meat was assumed to be eaten cooked. Finally, risk management strategies based on the effect of setting various target levels from prevalence distributions and their effect on POs were also assessed. The number of samples as well as the proportion of the acceptable contaminated units by the producer (ALS 95% CL), was calculated according to the mathematical procedures described by Whiting et al. (2006) and Van Schothorst et al. (2009).

3. Results and discussion

3.1. Prevalence of Salmonella

Salmonella was detected in seven out of the ten lots tested and, whenever present, the pathogen survived along the product shelf life, apart from lot No. 7, where only one positive sample was detected in S1. The number of positives detected in the different evaluated stages is shown in Fig. 1. Lots No. 1, 2, 4 and 6 were heavily contaminated in samples analyzed under S1, since positives ranged between 8 (67%) and 12 (100%). For lots No. 1, 2, 4 and 6 prevalence was maintained also under S2. Under S2 all samples were detected as positive in lot No. 5. Regarding S3, lots No. 1, 2, and 4 showed more than 50% positive samples and for S4, lots No. 1, 2 and 6 showed the highest number of *Salmonella* positives. This variability in lot contamination might reflect