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Microbiological criteria for *Campylobacter* in broiler carcasses in Italy: A possible approach to derive them

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ABSTRACT

The aim of this paper was to provide suitable microbiological criteria (MC) for *Campylobacter* in broiler carcasses and a sampling plan to verify compliance with such criteria. Data were gathered in the presence and concentration of *Campylobacter* in broiler carcasses collected in three different Italian slaughterhouses, labelled as A, B and C. The sampling plan to be validated in each slaughterhouse included the analysis of three different carcasses collected immediately after chilling from 30 different lots, for a total of 90 samples per slaughterhouse. The number of positive samples containing above 100 CFU/g and above 1000 CFU/g throughout the 30 tested lots was determined to estimate between-lot variability. Based on this information, the performance of four MC was evaluated for lot compliance: i) $n = 3$; $c = 0$; $m = 100$ CFU/g; ii) $n = 3$; $c = 0$; $m = 1000$ CFU/g; iii) $n = 3$; $c = 1$; $m = 1000$ CFU/g and iv) $n = 3$; $c = 2$; $m = 1000$ CFU/g. Positive *Campylobacter* samples were found in 60% of the lots tested in slaughterhouses A and C and in 73.3% of lots from slaughterhouse B. The differences among the three slaughterhouses in the mean *Campylobacter* levels found in positive samples were not significant and were used to evaluate the performance of the MC. The level of lot compliance to different MC was calculated and for the most stringent one ($n = 3$; $c = 0$; $m = 100$ CFU/g) was 40% at slaughterhouses A and C but only 26.7% at slaughterhouse B. The results of this study show an alternative approach to establish MC for *Campylobacter* in broilers. According to (1) *Campylobacter* prevalence and concentration in Italy, (2) applied experimental plan and (3) selected slaughterhouses, the number of compliant lots to the suggested MC ranged between 26.7 and 100%. The selection of the fit for purpose MC is a risk manager decision, based on a reasonable balance between public health and cost for poultry industries.

1. Introduction

Foods originating from animals are important sources of infections with a variety of pathogenic bacteria. In recent years, concerns about meat and meat products carrying foodborne pathogenic microorganisms have increased, despite improved efforts in meat and processed meat hygiene (Bae et al., 2011; Choi et al., 2009). All fresh meat can contain some degree of microbial contamination including pathogenic bacteria of animal and human origin (Cárdenas et al., 2008).

Handling, preparation and consumption of broiler meat may account for 20 to 30% of the 220,209 human cases of Campylobacteriosis reported in the European Union by 27 member states in 2011 (EFSA, 2013). Campylobacteriosis is the most frequently reported foodborne illness in the EU and the actual number of cases is believed to be around nine million each year. The cost of Campylobacteriosis to public health systems and to lost productivity in the EU is estimated by EFSA to be around EUR 2.4 billion a year (http://www.efsa.europa.eu/en/topics/topic/campylobacter.htm).

Countries have traditionally attempted to improve food safety by setting microbiological criteria for raw or finished processed products. Microbiological criteria define the acceptability of a product or a food lot based on the absence/presence or number of microorganisms, including parasites, and/or quantity of their toxins/metabolites per unit(s) of mass, volume, area or lot (CAC, 2013; ICMSF, 2002). Microbiological criteria are useful for validating and verifying HACCP-based processes and procedures. In EU legislation, they are also used as a method of communicating the level of hazard control that should be achieved. Microbiological criteria may be used by competent authorities and food business operators to achieve the Appropriate Level of Protection (ALOP), either directly or through other microbiological

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risk management metrics, such as Food Safety Objectives (FSOs) and Performance Objectives (POs) (CAC, 2013).

At present, neither microbiological criteria nor targets in primary production have been defined for *Campylobacter*. On 27 January 2005, the Scientific Panel on Biological Hazards of the European Food Safety Authority (EFSA) adopted an opinion encouraging the setting and use of performance objectives/targets for *Campylobacter* in poultry production. It stated that reducing the proportion of *Campylobacter* infected poultry flocks and/or reducing the number of *Campylobacter* in live poultry and on poultry carcasses would lower the risk to consumers considerably (Rosenquist et al., 2003).

The aim of this paper was to provide suitable microbiological criteria (MC) for *Campylobacter* in broiler carcasses and a sampling plan to determine compliance of broiler lots processed in different slaughterhouses with such criteria. The effect of slaughterhouse processing capacity on lot compliance was investigated and is discussed hereunder.

2. Materials and methods

2.1. Sampling

The study took place in Italy between June and September 2013 and was conducted at broiler-lot level in three different slaughterhouses, focusing on birds entering the food chain. The three slaughterhouses were selected in order to estimate the impact of the sampling plan described below in different productive settings. Two slaughterhouses, labelled as A and B, were big industrial slaughterhouses, working heavy (mean ± relative standard error = 3.75 ± 0.18 kg) and light chickens (i.e. mean ± relative standard error = 1.56 ± 0.06 kg), respectively. The third slaughterhouse, labelled as C, was smaller and slaughtered both light and heavy chickens (mean ± relative standard error = 2.09 ± 0.59 kg).

The sampling plan to be validated in each slaughterhouse included the analysis of three different carcasses collected from 30 different lots for a total of 90 samples per slaughterhouse. The three analysed carcasses were distributed approximately at the beginning, in the middle and at the end of the processed lot. This number was chosen to provide a realistic approach about the feasible sampling plan to be applied in a slaughterhouse. The whole number of samples per slaughterhouse was considered sufficient to build a statistical distribution representing microbial contamination. The sampling of carcasses and broiler lots was by random selection at the slaughterhouse level, and was distributed across different working days during the sampling week. As a general rule, Friday and days preceding national holidays were excluded. Samplings were performed in accordance with the European baseline survey on the prevalence of *Campylobacter* in broiler lots and on broiler carcasses, conducted in the EU in 2008 (European Food Safety Authority, 2010a, 2010b).

2.2. Detection of thermotolerant Campylobacter

From each randomly selected carcass, a sample of at least 35 g of neck and breast skin, avoiding any fat, was collected immediately after chilling but before freezing, cutting or packaging, and sent to the laboratory for detection and enumeration (determination of counts) of *Campylobacter*. Skin samples from broiler carcasses were collected immediately after chilling because the microbiological criteria were assumed to be set for carcasses collected immediately after chilling, as provided for in Regulation (EC) 2073/2005 for the detection of *Salmonella*.

All samples were analysed by the laboratory within 24 h of carcass collection time. *Campylobacter* organisms were isolated from skin samples as described in ISO 10272-1:2006(E) 'Microbiology of food and animal feeding stuffs—Horizontal method for detection and enumeration of *Campylobacter* spp. Part 1: Detection method'. The quantitative analysis of *Campylobacter* was carried out according to ISO/TS 10272-2:2006 'Microbiology of food and animal feeding stuffs—Horizontal method for detection and enumeration of *Campylobacter* spp. Part 2: Colony-count technique'. All lots where enumeration data were obtained and where *Campylobacter* was detected by the presence/absence on the carcasses were assumed to be truly positive. The theoretical limit of detection was considered to be 1 CFU/35 g sample (0.029 CFU/g) while the limit of quantification was set at 10 CFU/g.

2.3. Data processing

To start processing data, the percentage of positive and negative samples (considering a limit of quantification (LOQ) of 100 CFU/g) was initially calculated from each studied slaughterhouse. Samples yielding enumeration data were log-transformed and descriptive statistics (mean, standard deviation, median, percentiles 5th and 95th and maximum/minimum values) subsequently calculated (within-lot variability). For descriptive statistics calculations, the whole data were included in the analysis to get a better representation of the tested lots per slaughterhouse. To provide an estimate of between-lot variability, the number of positive samples above the LOQ and above 1000 CFU/g was determined throughout the 30 tested lots. This information was used to evaluate the performance of four microbiological criteria for lot compliance: i) $n = 3$; $c = 0$; $m = 100$ CFU/g; ii) $n = 3$; $c = 0$; $m = 1000$ CFU/g; iii) $n = 3$; $c = 1$; $m = 1000$ CFU/g and iv) $n = 3$; $c = 2$; $m = 1000$ CFU/g, where n is the number of units comprising the sample; c is the number of sample units giving values over m which is the microbiological limit. ANOVA analysis ($P < 0.05$) was conducted to study significant differences between the three slaughterhouses. All calculations were performed in MS Excel (Microsoft Corporation).

3. Results and discussion

3.1. Prevalence and concentration of Campylobacter on carcasses

Table 1 summarizes the results obtained from the microbial analyses to detect *Campylobacter* on carcasses and lots in the three poultry slaughterhouses. Overall, 43 (47.8%), 59 (65.6%) and 41 (45.6%) of the samples tested at slaughterhouses A, B and C, respectively, were positive for *Campylobacter* spp. Positive samples were found in 60% of lots tested at slaughterhouses A and C and in 73.3% of lots tested at slaughterhouse B.

The levels of *Campylobacter* in the positive skin samples are summarized in Table 2. Only two samples from slaughterhouse B and one from slaughterhouse C showed contamination levels higher than 10^4 CFU/g. Moreover, 6.7%, 28.9% and 10% of samples from slaughterhouses A, B and C, respectively, showed contamination levels between 10^3 and

Table 1
Campylobacter detection on skin samples and lots.

Slaughterhouses	Skin samples ($n = 90$)			Lots ($n = 30$)		
	Negative	Positive	>10^4 CFU/g	Negative	Positive	>10^4 CFU/g
A	47 (52.2%)	43 (47.8%)	0	12 (40%)	18 (60%)	0
B	31 (34.4%)	59 (65.6%)	2 (2.2%)	8 (26.7%)	22 (73.3%)	1 (3.3%)
C	49 (54.4%)	41 (45.6%)	1 (1.1%)	12 (40%)	18 (60%)	0