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ORIGINAL ARTICLE

Monitoring *Salmonella*, *Campylobacter*, *Escherichia coli* and *Staphylococcus aureus* in traditional free-range 'Label Rouge' broiler production: a 23-year survey programme

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Abstract

Aim: 'Label Rouge' broiler free-range carcasses have been monitored since 1991, and broiler flocks since 2010, for contamination by the main foodborne zoonotic bacteria.
Methods and Results: Initially, the monitoring plan mainly focused on the surveillance of *Salmonella*, and on indicators of the overall microbiological quality of free-range broiler carcasses such as *Staphylococcus aureus* and coliforms, but was extended in 2007 to include *Campylobacter* enumeration on carcasses and in 2010, to *Salmonella* in the environment of live birds. *Salmonella* contamination of free-range broiler carcasses rose to a peak of 16% in 1994 but less than 1% of carcasses are now regularly found to be positive. Indicators of the overall microbiological quality of carcasses are also improving. These results correlate with the low prevalence of *Salmonella* in free-range broiler breeding and production flocks, and with the continuous improvement of hazard analysis and critical control points in slaughterhouses, the implementation of a good manufacturing practice guide since 1997 and the application of EU regulations on *Salmonella* since 1998 in France. Regarding *Campylobacter* counts on carcasses, the situation has been improving continuously over the last few years, even if 2·5% of the carcasses are still contaminated by more than 1000 *Campylobacter* per g of skin.
Conclusions: Although the current control system focusing on *Salmonella* is based on firm epidemiologic data and offers effective means of control (e.g. slaughtering of positive breeder flocks), existing information on *Campylobacter* makes it more difficult to formulate an effective control plan for free-range broilers, due to their particular exposure to environmental contamination.
Significance and Impact of the Study: This long-term surveillance programme provided an extended view of the evolution of the contamination of free-range broilers and a direct measurement of the impact of mandatory and profession-driven interventions on the microbiological quality of carcasses.

Introduction

In Europe, *Campylobacter* and *Salmonella* have been identified as the two main agents causing food-borne illnesses (EFSA and ECDC 2015).

Recent publications by Batz *et al.* (2012) and Havelaar *et al.* (2012) ranked the disease burden of pathogens in food sources in the USA and Europe on the basis of estimates of the cost of illness and loss of quality-adjusted life years. These studies both concluded that '*Campylobacter* (poultry)' and '*Salmonella* (poultry)' are ranked in the top five food–pathogen combinations. In France, a recent publication by Van Cauteren *et al.* (2015) suggested that the estimated community incidence rate of

Campylobacteriosis is 852 cases per 100 000 inhabitants (90%, credible interval: 525–1690), a total 115 times higher than the number of cases actually reported. As such a situation is clearly troubling, it would be useful to implement appropriate actions to monitor and control these micro-organisms. European Union Member States implemented a Regulation (2160/2003/EC) and a Directive (2003/99/EC), whose purposes are respectively to establish ways of combating and controlling food-borne illnesses of animal origin. Although the control system currently focused on *Salmonella* is based on firm epidemiological data and offers effective control options, the lack of efficient measures against *Campylobacter* makes it more difficult to formulate an effective control plan. As published by EFSA & ECDC (2015), recent annual data showed a continuous decrease of Salmonellosis in humans while Campylobacteriosis has increased continuously since 2008.

Although the substantial decrease in the contamination of laying hens by *Salmonella* Enteritidis and Typhimurium is primarily responsible for the reduction of human infections, improved monitoring of broiler flocks to detect infection has also contributed significantly to these results. Meanwhile, the policy pursued by the EU and the Member States has failed to decrease the prevalence of Campylobacteriosis in humans. While it is unrealistic to foresee the eradication of *Campylobacter* in broiler flocks, as *Campylobacter* is generally considered to be a normal inhabitant of the chicken gut, the percentage of heavily contaminated broiler carcasses reduction (i.e. >1000 CFU g^{-1} of skin) should be considered as an option for combating Campylobacteriosis in humans (Romero-Barrios *et al.* 2013).

Although it accounts for 60% of the consumption of whole broiler carcasses and 15% of production in France, the development of free-range production remains low in other countries and few studies have been published regarding contamination of free-range birds by the main foodborne bacteria. Pieskus *et al.* (2008), in a survey carried out in four European countries (Italy, Lithuania, the Netherlands, Germany) of 196 flocks (2370 samples) of both free-range and standard production broilers, found a flock prevalence of *Salmonella* in standard production of 29% in Lithuania, 20% in Italy, 11% in the Netherlands and 0% in Germany, while the free-range flock prevalence was 1% in Italy and 7% in the Netherlands (no free-range flocks tested in Lithuania and Germany). In the Basque Country (Spain), Esteban *et al.* (2008) investigated 60 flocks from 34 free-range broiler farms and isolated *Campylobacter* in 70·6% (CI: 61·8–79·4) of the farms while *Salmonella* was present in only 2·9% (CI: 0·0–6·2). In Chile, in a survey made on 50 free-range broiler carcasses, Rivera *et al.* (2011) reported 34% *Campylobacter* prevalence (no counting done). In the USA, Thanissery *et al.* (2012) isolated *Salmonella* from 50% to 100% of free-range broiler carcasses and *Campylobacter* from 95% to 100% of the carcasses tested. In Belgium, on a longitudinal survey on six free-range farms, Vandeplas *et al.* (2010) found 100% *Campylobacter* prevalence during the summertime, 66·7% in spring and 33·3% in winter within the farms and their environment. In Greece, Economou *et al.* (2015) collected meat samples at slaughter from standard and free-range farms and found no significant difference in *Campylobacter* contamination between the two types of production (28·7% *vs* 29·4%). In the UK, Allen *et al.* (2011) isolated *Campylobacter* from 95% to 100% of the 28 organic and free-range flocks examined, while the contamination of standard flocks within the same time period was approximately 55%. Despite this result, the mean level of *Campylobacter* healthy caecal carriage by individual positive birds was the same disregarding the production system (6·2 to 6·7 $\log_{10}$ g^{-1}). In South Africa, Bester and Essack (2012) compared the level of *Campylobacter* caecal carriage in different traditional and industrial production systems in their country. The prevalence of *Campylobacter* was 68% in rural backyards, 47% in commercial free-range flocks, 47% in industrial standard broilers and 94% in industrial laying hens. All these studies showed that most of the time, while *Salmonella* prevalence was lower in free-range birds than in standard production, the situation, is more contrasted regarding *Campylobacter* infection of birds or carcasses contamination.

This study is a product of 23 years from the microbiological quality monitoring of the French traditional free-range 'Label Rouge' broiler production organized by Synalaf (the National Union of French Poultry Producers, an inter-professional organization created in 1967 to represent the regional organizations of Label Rouge poultry and egg producers) and ANSES (French Agency for Food, Environmental and Occupational Health & Safety). The example of the monitoring and control of *Salmonella*, thermotolerant coliforms, *Staphylococcus aureus* and *Campylobacter* in traditional free-range 'Label Rouge' broiler flocks and carcasses will be given to illustrate the possible efficacy of mandatory and profession-driven policies in ensuring food safety during production. Initially focusing on *Salmonella* surveillance and indicators of overall microbiological quality of free-range broiler carcasses such as *S. aureus*, and thermotolerant coliforms, the monitoring plan has now also been extended to include the numeration of *Campylobacter* on carcasses (since 2007) and *Salmonella* on live birds (since 2010).

Free-range 'Label Rouge' poultry production is a traditional broiler-rearing system in France using slow-growing poultry strains, fed with a diet including 75% cereals

at least, spending half of their life with access to outside areas and slaughtered at 81 days minimum (Légifrance 2013). Under these particular rearing conditions, and considering the exposure to wild animals and environmental contamination, the microbiological quality of carcasses is a major concern of the Label Rouge charter governing traditional free-range poultry production in France, and specific good manufacturing practice and an HACCP (hazard analysis and critical control points) guide have been implemented since 1997.

Materials and methods

Sampling plan

For carcasses and cut-up pieces produced under 'Label Rouge', one bacteriological sampling was organized per month and per abattoir. Once every three month, sampling was performed by the official certification authority. All the abattoirs slaughtering 'Label Rouge' broilers in France were sampled following this protocol. These abattoirs were located in every regions of France. Each control consisted of five samples individually analysed, resulting in a minimum of 60 samples per abattoir per year. The sample size was chosen according to the requirements of the Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs.

For live broilers, sampling was done for each flock during the 6 weeks preceding slaughter with two moistened boot swabs (Sterisox; Sodibox, Pont C'Hoat, Nevez, France), pooled for further analysis. Faecal material was collected by the operator by walking with these boot swabs on their shoes on different areas of the poultry house.

Sample collection and analysis

For each sample series, five samples of neck skin (whole carcasses) or muscle (breast fillets) or muscle and skin (legs) were removed with sterile surgical pliers and knife. Regarding sampling for *Salmonella* detection, each sample was made of 10 g (from 1991 to 2006) or 25 g (since 2007) of neck skin, issued from a pool of three different carcasses from the same batch. Samples were collected in sterile polyethylene bags transported to the laboratory within a day. Each sample was individually analysed at the laboratory within 24 h of reception. At the laboratory, the samples were prepared (ISO 6887 parts 1 and 2) and analysed following the ISO or AFNOR standardized methods for counts of *S. aureus* (ISO 6888 Part 1) and thermotolerant coliforms (AFNOR V08-060) and for detection of *Salmonella* (ISO 6579 Part 1). Another series of five samples of 10 g of neck skin (years 2007 and 2008) or leg skin (years 2009 and following) was sampled for counting *Campylobacter*. This change in sampling methods was operated as leg skin gave a more accurate view of the potential exposure of the consumer while neck skin was most of the time removed at the end of the slaughtering process. Counting were performed using ISO 10272 Part 2 method and choosing Karmali agar or mCCDA (modified Charcoal Cefoperazone Deoxycholate Agar) (provider is at the own choice of the laboratory).

Boot swabs from environments containing live birds were analysed following the technique described in ISO 6579, Annex D.

As each laboratory providing results for the survey worked under accreditation (ISO 17025) the methods used by the laboratory may have change regarding the evolution of AFNOR and ISO standard methods.

Interpretation of results

Results were classified under the class values mentioned in Table 1. Percentages of samples in each class value were calculated.

For *Campylobacter* counts, percentages of samples giving results <100 CFU g^{-1}, $100 <$ CFU $g^{-1} < 1000$ and >1000 CFU g^{-1} were calculated.

Statistical analysis

Differences on percentage of positive samples or in percentage of samples within the different classes and between two consecutive years were analysed using chi-squared test. Significant differences at $P < 0{\cdot}01$ were noticed as '**' in the figures and at $P < 0{\cdot}05$ as '*'. No_n significant differences were noticed as 'ns'.

Results

Since 1991, the number of carcasses analysed each year has varied from 3321 to 7233 depending on the production of broiler carcasses by each slaughterhouse (see Fig. 1). Since 2010, two boot swabs were taken in each flock before slaughtering for *Salmonella* sampling (see Fig. 2). Results of the monitoring programme are presented in Figs 2–6.

Table 1 Class values

	Class 1	Class 2
Staphylococcus aureus	$<5 \times 10^3$	$>5 \times 10^3$
Thermotolerant coliforms	$<10^4$	$>10^4$
Salmonella	Negative	Positive

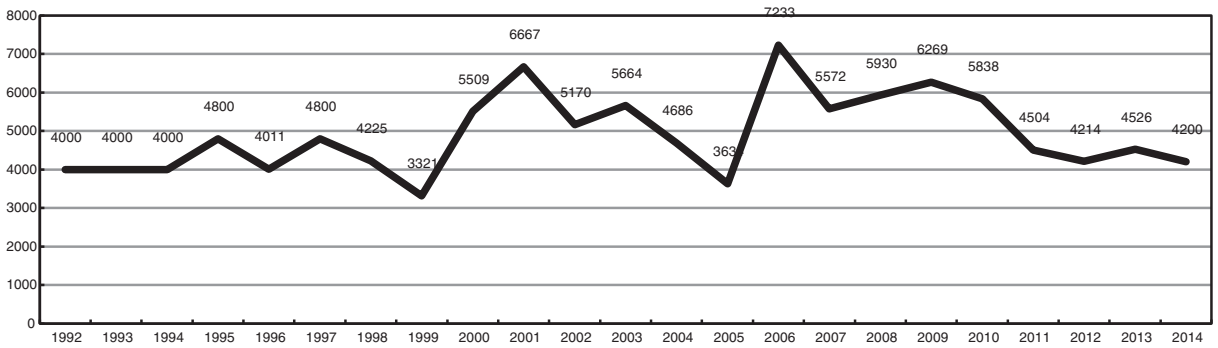

Figure 1 Number of carcass samples analysed from 1991 to 2014.

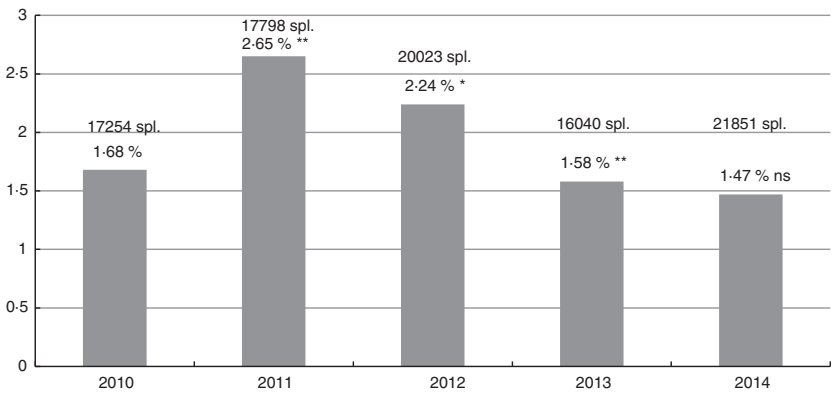

Figure 2 Percentages of *Salmonella* positive free-range broiler flocks from 2010 to 2014. *Significant difference with the previous year $P < 0{\cdot}05$. **Significant difference with the previous year $P < 0{\cdot}01$. ns, no significant difference with the previous year.

Salmonella on live birds

The overall prevalence of *Salmonella* on live birds (Fig. 2) has decreased over the years and is still decreasing, due probably to the improved monitoring of contamination in breeder flocks, and also to the duration of rearing of 'Label Rouge' broilers (81 days min.), which probably left time for the birds to develop a mature competitive exclusion flora (Humbert *et al.* 1989; Salvat *et al.* 1989) and an efficient immune response against *Salmonella*.

Salmonella on fresh broiler carcasses

As can be seen in Fig. 3, the overall contamination of 'Label Rouge' carcasses by *Salmonella* remained quite low over the years monitored (max. 16% in 1994 while the contamination of standard broilers carcasses the same year was over 50%, Salvat 2010), but dramatically decreased after 1999 when the European Directive 92/117/EC became mandatory in France (October 1998). A slight increase was observed in 2007 because of a change in the neck skin sampling (10 g before 2007, 25 g after). Since 2010, the contamination of carcasses has been comparable to those of live birds (see Figs 2 and 3). Thanks to slaughtering logistics ('Label Rouge' broilers were the first in the day to be slaughtered) cross-contamination could not be responsible for any increase in the contamination of 'Label Rouge' broiler carcasses.

Contamination of 'Label Rouge' poultry by *Staphylococcus aureus* and Coliforms

Figures 4 and 5 show the overall evolution of contamination of 'Label Rouge' broiler carcasses for the 23-year period. *Staphylococcus aureus* is considered as a good indicator of the cleaning and disinfection of slaughterhouses (especially plucking machines) while Coliform bacteria are a good indicator of the performance of evisceration machines, as most of them come from the gut (Lahellec *et al.* 1995). A regular decrease of unsatisfactory results has been observed since 1998, corresponding to the implementation of the good hygiene practice (GHP) guide for 'Label Rouge' slaughtering in 1997. Such a voluntary policy leads to less than 5% of unsatisfactory results, while more than 25% of carcasses were rated 'Synalaf Class 2' (see Table 1) in 1991.

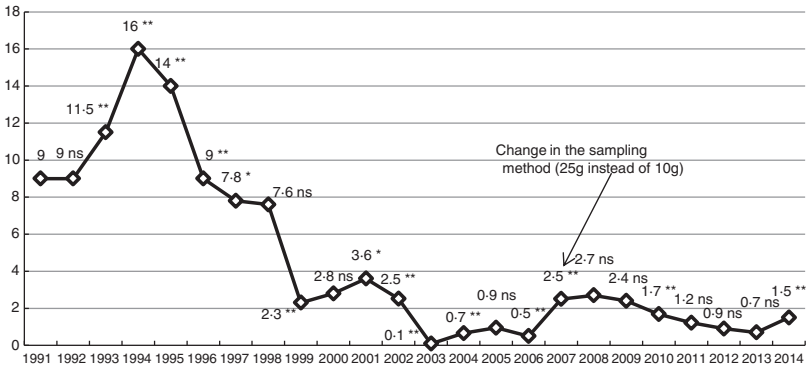

Figure 3 Percentages of *Salmonella*-contaminated 'Label Rouge' broiler carcasses from 1991 to 2014. *Significant difference with the previous year $P < 0{\cdot}05$. **Significant difference with the previous year $P < 0{\cdot}01$. ns, no significant difference with the previous year.

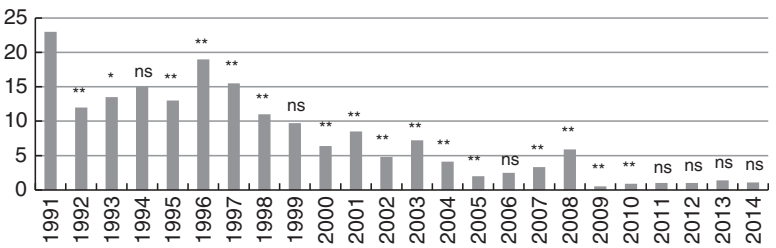

Figure 4 Evolution of the contamination of 'Label Rouge' broiler carcasses by *Staphylococcus aureus* from 1991 to 2014. *Significant difference with the previous year $P < 0{\cdot}05$. **Significant difference with the previous year $P < 0{\cdot}01$. ns, no significant difference with the previous year.

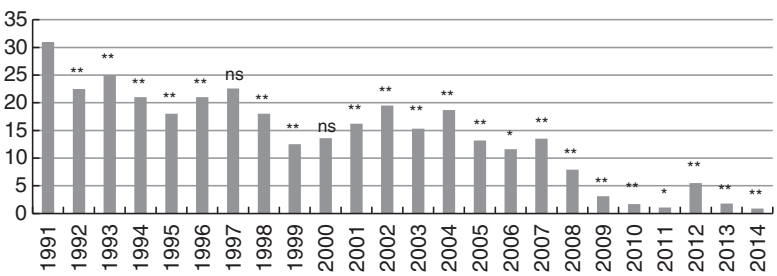

Figure 5 Evolution of the contamination of 'Label Rouge' broiler cracasses by Coliforms from 1991 to 2014. *Significant difference with the previous year $P < 0{\cdot}05$. **Significant difference with the previous year $P < 0{\cdot}01$. ns, no significant difference with the previous year.

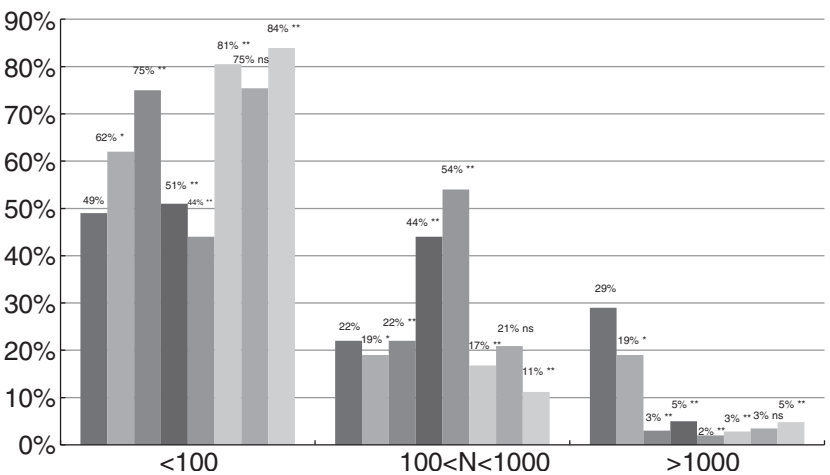

Figure 6 *Campylobacter* counts on 'Label Rouge' broiler carcasses from 2007 to 2014. *Significant difference with the previous year $P < 0{\cdot}05$. **Significant difference with the previous year $P < 0{\cdot}01$. Ns, no significant difference with the previous year. (■) 2007 (151 spl.); (■) 2008 (207 spl.); (■) 2009 (330 spl.); (■) 2010 (660 spl.); (■) 2011 (470 spl.); (■) 2012 (544 spl.); (■) 2013 (438 spl.); (■) 2014 (475 spl.).

Campylobacter on fresh broiler carcasses

Due to traditional free-range procedures and the long duration of their rearing, 'Label Rouge' broilers are particularly exposed to *Campylobacter* contamination during their lifetimes.

Figure 6 shows the evolution of *Campylobacter* counts since 2007 and shows that since that time, there has been

a considerable fall in the number of cases with the highest count (>1000 CFU g^{-1}), the category that may be responsible for cross-contamination during handling of carcasses by consumers (Fravalo *et al.* 2009). The highly significant decrease of heavily *Campylobacter*-contaminated carcasses noticed between year 2008 and 2009 was probably due to the change in sampling method: during years 2007 and 2008, neck skins were sampled while leg skins were sampled for year 2009 and following. Most carcasses nowadays are below 100 CFU g^{-1} and only 2–5% present more than 1000 CFU g^{-1}, which is to be compared with the results of an EFSA study in 2008 for standard broiler carcasses (EFSA 2010) where 21·6% of the *Campylobacter* count were >1000 CFU g^{-1} in the EU and 15·4% in France (neck skin samples).

Discussion

Salmonella contamination of Label Rouge broiler carcasses could nowadays be considered to be under control, as the contamination of broiler breeder flocks and of the environment remain low and comparable to or less than those observed by other authors (Esteban *et al.* 2008; Pieskus *et al.* 2008; Thanissery *et al.* 2012). The first decline of *Salmonella* contamination of broiler carcasses observed between years 1995 and 1998 could be associated with the implementation of the first French *voluntary* programme for *Salmonella* control in poultry based on the directive 92/117/EEC from the European council of 17 December 1992. The second major decrease occurred since 1999 and is probably associated to the implementation of the same European directive which became in 1998 *mandatory* in France. The change in sample size (10–25 g of neck skin since 2007 in order to follow the requirements of the Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs) had first a highly significant effect on the increase of the positive sample, but continuous progress in contamination of breeder flocks and on biosecurity measures over the period improved regularly the results since 2009. Despite that, a significant increase of the percentage of *Salmonella* positive carcasses was noticed in 2014 (0·7% in 2013 *vs* 1·5% in 2014), which could not be explained by an overall increase percentage of positive broiler flocks (see Figs 2 and 3). This increase in *Salmonella* positive broiler carcasses is mainly associated with *Salmonella* Indiana positive samples in three production organizations which were moderately contaminated by this serovar in their broiler flocks. Regarding the contamination of broiler carcasses by other micro-organisms such as *S. aureus* and thermotolerant coliforms, the implementation of HACCP and GHP in abattoirs since 1997 has improved the safety of carcasses, as shown by the more or less continuous decrease of heavily contaminated carcasses measured after the implementation of the Synalaf GHP guide.

The European situation is quite different for *Campylobacter* as all the publications on the contamination of free-range farms or carcasses showed heavily contaminated flocks in many countries (Esteban *et al.* 2008; Vandeplas *et al.* 2010; Allen *et al.* 2011; Rivera *et al.* 2011; Bester and Essack 2012; Thanissery *et al.* 2012; Economou *et al.* 2015). Many publications have investigated the risk factors of *Campylobacter* in poultry production. A recent critical review by Newell *et al.* (2011) found that while the season continued to be the main risk factor (summer, autumn), biosecurity (i.e. quality of drinking water, of cleaning and disinfection, duration of depopulation period between flocks, ventilation, protection against insects and rodents, slurry management, flock size, thinning, etc.) is the factor offering the greatest potential for control solutions. Free-range farming was also a main risk factor identified in many studies, and the age of the birds at slaughter is another (Newell *et al.* 2011). Regarding these risk factors, excluding thinning, which is forbidden for 'Label Rouge' broilers in France, free-range rearing was a critical situation as birds are in contact with an open environment, which is considered as the main source of broiler exposure to *Campylobacter* infection (Huneau-Salaün *et al.* 2007) and are slaughtered at a minimum of 81 days. Indeed, contamination of poultry flocks by *Campylobacter* generally occurred late (after 15–20 days of rearing) and the gut flora of the young bird is able to provide good protection against *Campylobacter* colonization (Laisney *et al.* 2004). At slaughter, the main risk factors identified are linked to those monitored at the farm level such as, season, age at slaughter, thinning, etc. (Hue *et al.* 2010). A review of contamination at the abattoir (Rosenquist *et al.* 2006) showed that although scalding is a decontamination step, plucking and evisceration were the main contamination steps. Further steps such as washing and chilling are cleansing operations and therefore less significant. Despite that, high levels of *Campylobacter* such as more than 10^3 *Campylobacter* per g may still be found at the end of the process (Rosenquist *et al.* 2006) providing a potentially infective dose available for further cross-contaminations once in the possession of the consumer. The percentage of samples presenting a count higher than 10^3 CFU g^{-1} decreased during the last 6 years of the survey (from 29% to less than 5%), but the main decrease observed between year 2008 and years 2009 and following, is mainly associated with a change in the sampling method, as neck skin samples were replaced by leg skin samples which was supposed to be less contaminated by the

processing operations. While eradication of *Campylobacter* in poultry is illusory considering the high affinity of the bacterium for bird gut microbiota, reducing the risk for humans is possible because there is a close relationship between contamination of carcasses and contamination of the caeca (Reich *et al.* 2008). As chemical decontamination of carcasses is forbidden in Europe, and considering the inability of *Campylobacter* to grow in food, a strategy of reducing caecal colonization may succeed in reducing the risk for humans. A study carried out by EFSA (Romero-Barrios *et al.* 2013) in four EU Member States tried to evaluate the performance of different methods at the farm level (for standard broilers) and at the slaughterhouse, regarding their potential efficacy in reducing risk for consumers. The authors recommended that the following control measures should be implemented to reduce foodborne diseases in humans:

i Application of strict biosecurity measures: 16% reduction of foodborne Campylobacteriosis (some of them not applicable for free-range production during the outdoor period).
ii Use of fly nets: 60% reduction of foodborne Campylobacteriosis (1 country: Denmark) (not applicable for free-range production)
iii Avoid thinning: 1·8–25% reduction of foodborne Campylobacteriosis (forbidden in Label Rouge production)
iv Decreased slaughter age (Label Rouge broilers are slaughtered at a minimum of 81 days):

- at 42 days: 0–5% reduction of foodborne Campylobacteriosis
- at 35 days: 0·6–18% reduction of foodborne Campylobacteriosis
- at 28 days: 21–43% reduction of foodborne Campylobacteriosis

v Reduction of caecal contamination:

- 1 $\log_{10}$ reduction: 48–83% reduction of foodborne Campylobacteriosis
- 2 $\log_{10}$ reduction: 76–98% reduction of foodborne Campylobacteriosis
- 3 $\log_{10}$ reduction: 90–100% reduction of foodborne Campylobacteriosis
- 6 $\log_{10}$ reduction: 100% reduction of foodborne Campylobacteriosis.

The most efficient measures are those impacting the number of *Campylobacter* in caeca, but methods available for the purpose at the present time, such as bacteriophages (Wagenaar *et al.* 2005; El-Shibiny *et al.* 2009), vaccination (De Zoete *et al.* 2007; Buckley *et al.* 2010; Neal-McKinney *et al.* 2014), and the use of probiotics, prebiotics, bacteriocins or short chain fatty acids (Solis De Los Santos *et al.* 2008, 2009; Willis and Reid 2008; Rihakova *et al.* 2009; Svetoch and Stern 2010; Van Gerwe *et al.* 2010; Guyard-Nicodeme *et al.* 2016) need to be validated. Until then, the best way to prevent consumer infections remains the application of GHPs in the kitchen during preparation of food.

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Conflict of Interest

No conflict of interest declared.

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