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SCIENTIFIC OPINION

Scientific Opinion on *Campylobacter* in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain[1]

EFSA Panel on Biological Hazards (BIOHAZ)[2, 3]

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ABSTRACT

It is estimated that there are approximately nine million cases of human campylobacteriosis per year in the EU27. The disease burden of campylobacteriosis and its sequelae is 0.35 million disability-adjusted life years (DALYs) per year and total annual costs are 2.4 billion €. Broiler meat may account for 20% to 30% of these, while 50% to 80% may be attributed to the chicken reservoir as a whole (broilers as well as laying hens). The public health benefits of controlling *Campylobacter* in primary broiler production are expected to be greater than control later in the chain as the bacteria may also spread from farms to humans by other pathways than broiler meat. Strict implementation of biosecurity in primary production and GMP/HACCP during slaughter may reduce colonization of broilers with *Campylobacter*, and contamination of carcasses. The effects cannot be quantified because they depend on many interrelated local factors. In addition, the use of fly screens, restriction of slaughter age, or discontinued thinning may further reduce consumer risks but have not yet been tested widely. After slaughter, a 100% risk reduction can be reached by irradiation or cooking of broiler meat on an industrial scale. More than 90% risk reduction can be obtained by freezing carcasses for 2-3 weeks. A 50-90% risk reduction can be achieved by freezing for 2-3 days, hot water or chemical carcass decontamination. Achieving a target of 25% or 5% BFP in all other MS is estimated to result in 50% and 90% reduction of public health risk, respectively. A public health risk reduction > 50% or > 90% could be achieved if all batches would comply with microbiological criteria with a critical limit of 1000 or 500 CFU/gram of neck and breast skin, respectively, while 15% and 45% of all tested batches would not comply with these criteria.

KEY WORDS

Broiler meat, *Campylobacter*, campylobacteriosis, control, microbiological criteria, QMRA, targets

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SUMMARY

Following a request from the European Commission, the Panel on Biological Hazards was asked to deliver a scientific opinion on *Campylobacter* in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain. EFSA commissioned the development of a quantitative microbiological risk assessment (QMRA) model which has been used to estimate the impact on human campylobacteriosis due to the presence of *Campylobacter* spp. in broiler meat. This QMRA was also used to rank/categorize selected intervention strategies in the farm to fork continuum, for which quantitative data, of sufficient quality on efficacy for *Campylobacter* reduction at the point of application, were available.The evaluation of microbiological criteria required the development of a specific model by the BIOHAZ Panel, using data from the EU baseline survey, as an input.

It is estimated that there are approximately nine million cases of human campylobacteriosis per year in the EU27. The disease burden of campylobacteriosis and its sequelae is 0.35 million disability-adjusted life years (DALYs) per year and total annual costs are 2.4 billion €.

Campylobacter jejuni and *C. coli* are considered equivalent for the purpose of risk assessment in this opinion because there is no information on variability between these two species with respect to their behaviour in the food chain, impact of interventions or virulence for humans. There are no indications that *Campylobacter* strains with antimicrobial resistance behave differently in the food chain than their sensitive counterparts.

As previously estimated by the BIOHAZ Panel, handling, preparation and consumption of broiler meat may account for 20% to 30% of human cases of campylobacteriosis, while 50% to 80% may be attributed to the chicken reservoir as a whole (broilers as well as laying hens). The transmission routes from chickens to humans, other than handling, preparation and consumption of broiler meat, are not well understood, and related public health benefits cannot currently be quantified.

The public health benefits of controlling *Campylobacter* in primary broiler production are expected to be greater than control later in the chain as the bacteria may also spread from farms to humans by other pathways than broiler meat. There is, however, very little information about these pathways and quantifying the impact of interventions at farm level was only done for broiler meat-related cases.

Strict implementation of biosecurity in primary production and of GMP/HACCP during slaughtering is expected to reduce the level of colonization of broilers with *Campylobacter*, and the contamination level of carcasses and meat from colonized flocks. The effects of such implementation cannot be quantified because they depend on many interrelated local factors. Nevertheless, their impact on public health risk reduction may be considerable.

Quantitative risk assessment based on data from four countries has concluded that there is a linear relationship between prevalence of *Campylobacter* in broiler flocks and public health risk. The risk reduction associated with interventions in primary production is expected to vary considerably between MSs. Reducing the numbers of *Campylobacter* in the intestines at slaughter by 3 $\log_{10}$-units, would reduce the public health risk by at least 90%. Reducing the numbers of *Campylobacter* on the carcasses by 1 $\log_{10}$-unit, would reduce the public health risk by between 50 and 90%. Reducing counts by more that 2 $\log_{10}$ units would reduce the public health risk by more than 90%. The risk reduction associated with reducing concentrations on carcasses is expected to be similar in all MSs (although the baseline level of risk differs considerably).

Vertical transmission does not appear to be an important risk factor for colonization of broiler chickens with *Campylobacter*. Logistic slaughter, the separate slaughter, dressing and processing of negative and positive flocks, has negligible effect on human health risk.

Based on the results of the quantitative risk assessment, the specific control options discussed in this opinion would reduce public health risk is as follows:

In primary production, based on the results of one MS, 50-90% risk reduction can be achieved by the use of fly screens in the presence of strict biosecurity measures. Based on the results of four countries, up to 50% risk reduction can be achieved by restriction of slaughter age of indoor flocks to a maximum of 28 days, and up to 25% risk reduction by discontinued thinning.

After slaughter, a 100% risk reduction can be reached by irradiation or cooking on an industrial scale, if re-contamination is prevented. More than 90% risk reduction can be obtained by freezing carcasses for 2-3 weeks. A 50-90% risk reduction can be achieved by freezing for 2-3 days, hot water carcass decontamination or chemical carcass decontamination with lactic acid, acidified sodium chlorite or trisodium phosphate.

Scheduled slaughter aims to identify colonized flocks before slaughter so that they can be subjected to decontamination treatment. In low prevalence situations, the number of batches that need treatment is strongly reduced. Risk assessment, based on data from two countries, indicated that, when testing four days before slaughter, 75% of the colonized flocks are detected.

Control options in primary production, such as restriction of slaughter age and discontinuing thinning are directly available from a technical point of view but interfere strongly with current industrial practices. Control options for reducing carcass concentration, such as freezing, hot water and chemical decontamination are also directly available. Chemical decontamination is subject to approval in the EU and no chemicals are currently approved for use.

The BIOHAZ Panel has evaluated the public health risk reduction of applying instruments that are currently available in EU legislation, i.e. targets at primary production and microbiological criteria for foodstuffs.

Assuming all countries with a between-flock prevalence (BFP) of less than 25% or 5% in 2008 maintain that status, then achieving a target of 25% or 5% BFP in all other MS is estimated to result in 50% and 90% reduction of public health risk at the EU level, respectively. The realistic time period needed to obtain reductions due to targets in primary production will differ between countries depending on the present status and possibilities for practical implementation of different interventions and is a risk management issue. It is not realistic to consider targets for flocks with outdoor access. The share of such flocks in the total production is expected to increase in future.

The public health benefits of setting microbiological criteria were evaluated using data from the 2008 EU baseline survey. These estimates are average values for the whole EU; the impact could be very different between MSs. Theoretically, a public health risk reduction > 50% or > 90% at the EU level could be achieved if all batches that are sold as fresh meat would comply with microbiological criteria with a critical limit of 1000 or 500 CFU/gram of neck and breast skin, respectively. Correspondingly, a total of 15% and 45%, of all batches tested in the EU baseline survey of 2008, would not comply with these criteria.

Microbiological criteria could theoretically be implemented immediately but the ability to comply will also differ between MSs. They stimulate improved control of *Campylobacter* during slaughter.

The BIOHAZ Panel recommended that effective control options should be selected and verified under conditions where the application is intended to be used by industry to reduce *Campylobacter* and comply with potential targets and/or MC when established. Several data gaps were identified and generation of data in several areas was recommended.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

A total of 175,561 human cases of campylobacteriosis were reported in the EU25 in 2006, being the most frequently reported zoonosis. Poultry meat is considered to be a major source of infection. It can infect people through cross-contamination to ready-to-eat foods and direct hand-to-mouth transfer through food preparation, and to a lesser extent from the consumption of undercooked poultry meat.

On 27 January 2005, the Scientific Panel on Biological Hazards adopted an Opinion at the request of the Commission on *Campylobacter* in animals and foodstuffs[4]. The Opinion encourages the setting and use of performance objectives/targets in poultry production. It states that "Reducing the proportion of *Campylobacter* infected poultry flocks and/or reducing the number of *Campylobacter* in live poultry and on poultry carcasses will lower the risk to consumers considerably". Setting microbiological criteria for *Campylobacter* in poultry meat products at retail level appeared not to be cost effective.

In accordance with Commission Decision 2007/516/EC[5], all Member States carried out a harmonised baseline survey on the prevalence and antimicrobial resistance of *Campylobacter* spp. in broiler flocks and broiler carcases. On carcases, both qualitative and quantitative analyses were performed. The survey provides reference values, comparable between Member States, in order to consider future performance objectives/targets along the broiler meat production chain.

The Commission has two legal bases to consider performance objectives/ targets:

In accordance with Regulation (EC) No 2160/2003 on the control of *Salmonella* and other specified food-borne zoonotic agents[6], targets for the reduction of the prevalence of *Campylobacter* can be adopted at the level of primary production and, where appropriate, at other stages of the food chain.

In accordance with Regulation (EC) No 852/2004 on the hygiene of foodstuffs[7], microbiological criteria can be adopted for broiler meat. Similar to the provisions in Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs[8], both food safety and process hygiene criteria may be considered.

Before deciding on risk management measures and setting performance objectives / targets, the Commission may carry out a cost/benefit analysis. A quantitative assessment of the public health benefit and potential control options should therefore be carried out.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

EFSA is asked to further elaborate and update, in a quantitative way, its Opinion of the Scientific Panel on Biological Hazards related to *Campylobacter* in animals and foodstuffs, adopted on 27 January 2005 as regards broiler meat production. In particular, EFSA is asked to:

1. Identify and rank the possible control options within the broiler meat production chain (pre-harvest, at harvest and post-harvest), taking into account the expected efficiency in reducing human campylobacteriosis. Advantages and disadvantages of different options should be considered.

2. Propose potential performance objectives and/or targets at different stages of the food chain in order to obtain e.g. 50% and 90% reductions of the prevalence of human campylobacteriosis in the EU caused by broiler meat consumption or cross-contamination. The performance objectives might include targets for reduction at pre-harvest and/or microbiological criteria for foodstuffs

[4] The EFSA Journal (2005) 173, p. 1-10
[5] OJ L 190, 21.7.2007, p. 25.
[6] OJ L 325, 12.12.2003, p. 1.; Regulation as amended by Regulation (EC) No 1237/2007 (OJ L 280, 24.10.2007, p. 5).
[7] OJ L 139, 30.4.2004, p. 1, as corrected by OJ L 226, 25.6.2004, p. 3.
[8] OJ L 338, 22.12.2005, p. 1, Regulation as amended by Regulation (EC) No 1441/2007 (OJ L 322, 7.12.2007, p. 12)

(qualitative or quantitative criteria for *Campylobacter* in general or for certain strains (e.g. species, resistant to certain antibiotics)). In addition, guidance should be given on a realistic time period needed to achieve these reductions, taking into account the outcome of (1).

For the purpose of this mandate, broilers are defined as birds of the species *Gallus gallus*, specifically reared for the production of meat under various production systems.

ASSESSMENT

1. Introduction

In 2009, *Campylobacter* continued to be the most commonly reported gastrointestinal bacterial pathogen in humans in the EU since 2005. The number of reported confirmed human campylobacteriosis cases in the EU increased by 4.0 % in 2009 compared to 2008. The increase was also reflected as an increase in the overall EU campylobacteriosis notification rate, increasing from 43.9 per 100,000 population in 2008 to 45.6 per 100,000 population in 2009. The EU notification rate of confirmed cases of campylobacteriosis showed a slightly fluctuating, but stable, trend in the last five years (EFSA/ECDC, 2011). .

The genus *Campylobacter* currently comprises 23 species (2009) and this number is constantly increasing due to the identification of new species. Members of the genus are typically Gram-negative, non-spore-forming, S-shaped or spiral shaped bacteria. They are microaerophilic, but some can also grow aerobically or anaerobically. Thermophilic *Campylobacter* (*C. jejuni*, *C. coli*, *C. lari, C. upsaliensis* and *C. helveticus*) do not grow below 30°C and have an optimal growth temperature at 42°C. *Campylobacter* are sensitive to many external physical conditions like low water activity, heat, UV light and salt. In contrast to e.g. *Salmonella*, *Campylobacter* does not multiply outside a warm-blooded host (e.g. on meat samples) because of the absence of microaerobic conditions and non-permissive temperatures. The amplification vessel, and therefore the reservoir, of *Campylobacter* spp. are warm-blooded animals, including food-producing animals (cattle, sheep, pigs and poultry), wildlife and domestic pets. However, *Campylobacter* can survive in the environment especially when protected from dryness, which is one of the major stresses for this organism. Most surface water sources are contaminated by animal manure, containing *Campylobacter*. In slurries and in dirty water *Campylobacter* can survive for up to 3 months (Nicholson *et al.*, 2005).

Over 80% and approximately 10% of the human cases are caused by *C. jejuni* and *C. coli*, respectively. The species *C. jejuni* comprises two subspecies (*C. jejuni* subsp. *jejuni* and *C. jejuni* subsp. *doylei*). In routine diagnostics the two subspecies are rarely differentiated.

Other *Campylobacter* species, such as *C. upsaliensis, C. lari*, and *C. fetus*, may also be associated with human diarrhoea. The detection of non-*C. jejuni*/*coli* is uncommon in human cases in the industrialised world but more common in the developing world (Lastovica and Allos, 2008). This document focuses on *C. jejuni* and *C. coli* unless otherwise stated. No further differentiation between species or subtypes is made, as there is no pertinent information on differences in behaviour in the food chain, or with respect to virulence. This is further discussed in Chapter 5.3.2.3.

In susceptible humans, *C. jejuni*/*coli* infection is associated with acute enteritis and abdominal pain lasting for up to seven days or longer. Although such infections are generally self-limiting, complications can arise and may include bacteraemia, Guillain–Barré syndrome, reactive arthritis, inflammatory bowel disease, and irritable bowel syndrome (Gradel *et al.*, 2009; Haagsma *et al.*, 2006; Havelaar *et al.*, 2000; Helms *et al.*, 2003; Mangen *et al.*, 2005; Smith and Bayles, 2007).

Campylobacteriosis is largely perceived to be food-borne, with poultry meat as a major source. However, other sources have been identified (e.g. direct animal contact, environmental sources). A recently published Scientific Opinion of the Panel on Biological Hazards (EFSA, 2010d) assessed the extent to which poultry meat contributes to human campylobacteriosis. This Opinion estimates that the handling, preparation and consumption of broiler meat may account for 20% to 30% of human cases, while 50% to 80% may be attributed to the chicken reservoir as a whole. These results suggest that strains from the chicken reservoir may reach humans by pathways other than food (e.g. via the environment or by direct contact). Therefore, implementation of *Campylobacter* control measures at the primary production level is expected to result in a reduction of human exposure not only due to the reduction of contamination of broiler meat along the food chain but also to a lower exposure through pathways other than broiler meat. However, that Scientific Opinion (EFSA, 2010d) indicates that the results of the analysis should be interpreted with caution as they may be biased by inaccurate exposure

assessments and confounded by immunity. In addition bias may have resulted from the lack of representative typing data for isolates from humans and potential reservoirs in the different countries and regions of the EU, where the epidemiology of *Campylobacter* is believed to differ.

All types of poultry (e.g. broilers, layers, turkeys, ducks, geese, quails, ostriches) and wild birds can become colonized with *Campylobacter* (Newell and Wagenaar, 2000; Waldenstrom *et al.*, 2002). In contrast to *Salmonella*, the consumption of eggs does not contribute to the human campylobacteriosis problem as *Campylobacter* is rarely, if ever, transmitted vertically (Callicott *et al.*, 2005). However, the high numbers of *Campylobacter* in the intestinal tract of birds may contaminate the meat during processing and one important source for human exposure is considered to be cross-contamination either directly onto other foods or via the kitchen environment from poultry meat during food preparation.

1.1. Approach to responding to the terms of reference

To provide an indication of the potential health benefits of controlling *Campylobacter* on broiler meat, an updated estimate is provided on the incidence of human campylobacteriosis in the EU. This estimate is based on the comparative risks of Swedish travellers to EU Member States (MS) to be diagnosed with campylobacteriosis after return to their home country, using recent data.

The first term of reference requests EFSA to identify and rank possible control options for *Campylobacter* within the broiler meat production chain, specifically at pre-harvest, harvest and post-harvest. In this Opinion, the focus is on options to control *Campylobacter* in three steps of the food chain:

i. In primary production;

ii. During transportation and before slaughter;

iii. At slaughter, dressing and processing.

These are the steps that are most likely to be influenced by EU regulations. Control options at retail or during preparation and handling of broiler meat are not discussed.

For these steps, firstly descriptive information is provided on the current contamination of broiler and broiler meat with *Campylobacter* in the EU. This is followed by current knowledge of risk factors that influence the prevalence and level of contamination. To this end the EU-wide baseline study of 2008 provides important and timely information. This information is complemented by data from the peer-reviewed literature. Subsequently, interventions available to modify these risk factors, or otherwise reduce contamination, are discussed. The effects of selected interventions are then indicated as the expected reduction in prevalence and/or level of contamination at the point of intervention. Expert opinion was used to indicate the advantages and disadvantages of potential interventions. To indicate the time scale needed for interventions to be implemented, their current availability is indicated.

Quantitative microbial risk assessment (QMRA) is a well-recognised component of modern risk analysis and is used to estimate the impact of a particular hazard/product combination and/or changes in processing on public health. To date QMRA has been extensively used to investigate the role of broiler meat contamination in campylobacteriosis in national studies. These investigations are reviewed. However, for this Opinion a novel QMRA approach was commissioned by EFSA (CAMO), and has been used to estimate the impact on human campylobacteriosis (in particular mild to severe diarrhoea) due to the presence of *Campylobacter* spp. in broiler meat (carcasses and derived meat cuts) in four countries. This QMRA was also used to rank/categorize selected intervention strategies in the farm to fork continuum, for which quantitative data, of sufficient quality on efficacy for *Campylobacter* reduction at the point of application, were available. The results are presented as the expected percentage reduction of human health risk due to implementation of an intervention. No attempt was made to estimate the impact of combined interventions. Intra-community trade of broiler

meat, as well as import to or export from third countries, was not considered. Hence, the estimated reduction of human health risk is related to broiler meat produced and consumed in the EU. Finally a discussion of the validity and limitations of risk assessment modelling in general, and CAMO in particular, has been provided.

The second term of reference requests EFSA to identify potential performance objectives and/or targets to obtain 50% and 90% reductions of the prevalence of human campylobacteriosis. Throughout the opinion this is understood as a reduction of true (estimated) annual incidence of human campylobacteriosis associated with the preparation and handling of broiler meat. The BIOHAZ Panel, in accordance with the risk managers, has chosen to evaluate in particular the instruments that are currently available in EU legislation, i.e. targets at primary production and microbiological criteria for foodstuffs. The impact of applying such instruments at the level of individual Member States and for the EU as a whole is discussed. The quantitative evaluation of selected intervention targets in primary production is based on CAMO. However, the evaluation of microbiological criteria required the development of a specific model by the ad-hoc Working Group (CAMC), using data from the EU baseline survey as an input.

This Opinion only briefly discusses potential effects of strain differences with regard to outcomes of the risk assessment. Antimicrobial resistance is not considered separately, as there are no indications that resistant strains behave differently in the food chain compared to their sensitive counterparts. Hence, effect estimates of interventions in the food chain for sensitive and resistant strains will be similar. Furthermore, a recent review on quinolone resistant *Campylobacter* and other resistant bacteria in the food chain has concluded that "there are no indications that the disease burden has increased as a consequence of quinolone resistance and the healthcare costs are similar to those for susceptible *Campylobacter* infections" (Geenen *et al.*, 2011).

1.2. Underreporting of human campylobacteriosis in the EU

In 2008 (the baseline year for the quantitative risk assessment in this Opinion), there were approximately 191,000 notified cases of campylobacteriosis in the EU. The EU-average incidence rate of reported cases was 43.9 cases per 100,000 population (EFSA/ECDC, 2011). As discussed in a previous Opinion of EFSA (EFSA, 2010d), there is considerable under-ascertainment and under-reporting and the true incidence of human campylobacteriosis was estimated to range between 2 and 20 million cases per year. There is little information on specific under-reporting factors in different MSs. In order to compare the risks associated with the prevalence and numbers of *Campylobacter* on broiler carcasses, as identified in the EU baseline survey (EFSA, 2010a, 2010b), with human disease incidence, country-specific estimates of the true incidence of campylobacteriosis and the fraction attributable to the consumption and handling of chicken meat would be required. For the purpose of this opinion, the estimates on the true incidence were based on data describing differential risks to Swedish travellers as published originally by Ekdahl and Anderson (2004) and Ekdahl and Giesecke (2004). Updated information on the risk for Swedish travellers in the EU in the years 2005-2009 was obtained from the Swedish Institute for Communicable Disease Control (Smittskyddsinstitutet, SMI). The risk of campylobacteriosis in returning Swedish travellers in the EU was 15.9 per 100,000 journeys (90% CI: [15.6;16.2]), ranging between 0.40 for Finland to 182 for Bulgaria. Estimates of the true incidence were anchored to population-based estimates from the Netherlands, based on raw data from a Dutch case-control study (de Wit *et al.*, 2001), where the incidence rates from these studies were applied to the average population of 2005-2009 and scaled to the observed average of laboratory-confirmed cases for these years in comparison to the year 1999 when the case-control study was performed. An average of 81,300 (90% CI [29,900;173,000]) cases of campylobacteriosis per year was estimated to occur in the Netherlands between 2005 and 2009. Further details on the methods and results are provided in Appendix A.

For the EU-27, the estimated true incidence of campylobacteriosis is approximately 9.2 (90% CI [3.3;20]) million cases, which fits well in the range reported before. The underreporting factor at the EU level is 48.4 (90% CI [17;103]), implying that only 2.1% (90% CI [0.8;5]) of all cases is currently reported. The estimated true incidence rates per MS are visualised in Figure 1.

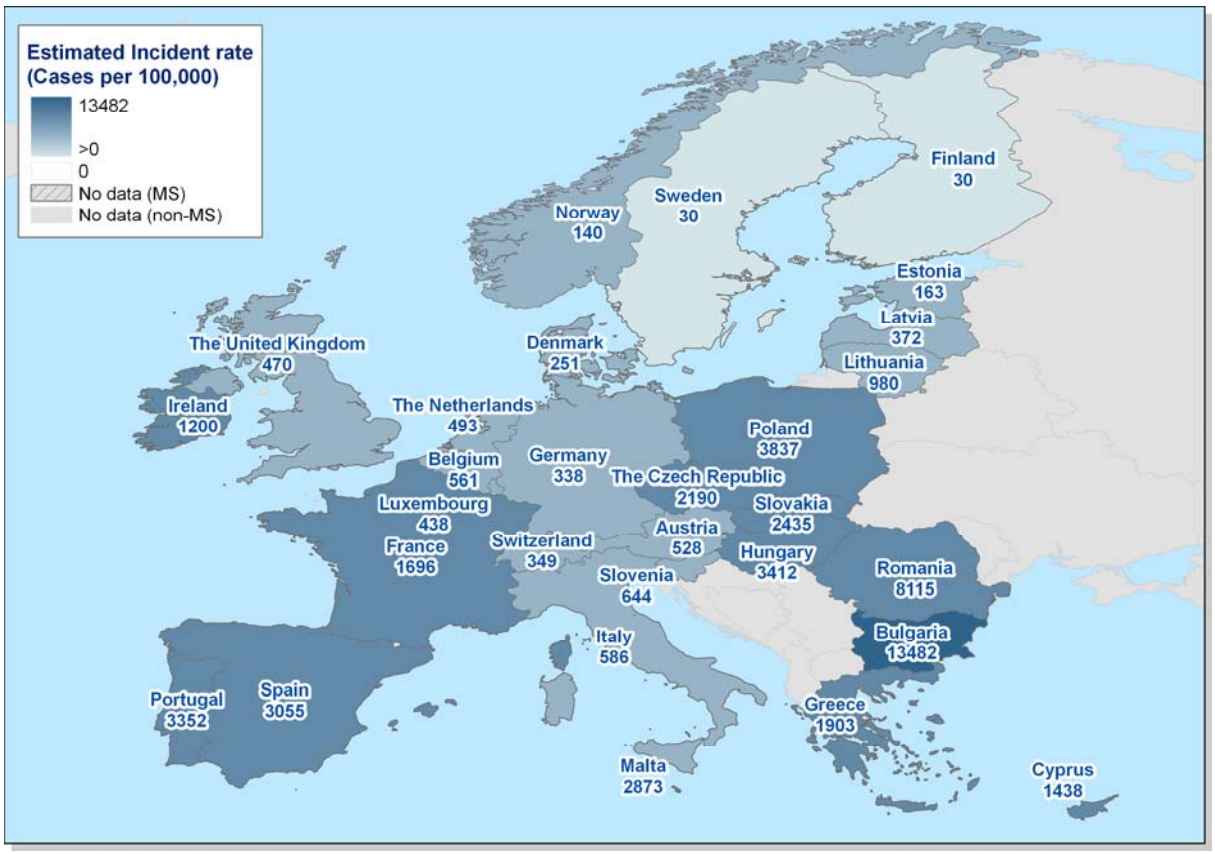


Figure 1: Estimated true incidence rate (per 100,000) of human campylobacteriosis in the EU-27.

The risks to Swedish travellers in the period 2005-2009 were similar to those reported by Ekdahl and Giesecke (2004) for the period 1997-2003. The estimated true incidence of campylobacteriosis was significantly correlated with a risk estimate for *Campylobacter* on broiler meat, as discussed in Chapter 6. This would imply that in countries with a high prevalence and concentration of *Campylobacter* on broiler carcasses, there is also a high risk of human campylobacteriosis.

These data suggest a very high incidence of human campylobacteriosis in the EU (each year, approximately one out of every 50 inhabitants would be affected) and an important contribution of contaminated broiler meat, in particular in high incidence countries. Consequently, considerable public health benefits are expected if the *Campylobacter* contamination of broiler carcasses is reduced. Nevertheless, the limitations and assumptions in the risk assessment models need to be taken into consideration when interpreting these findings.

The case data are extracted from the Swedish infectious disease surveillance system (SmiNet) and rely on laboratories and physicians reporting diagnosed cases to SMI. Clearly, only a fraction of all cases of illness are reported. Both, domestic and travel cases are counted as such, without consideration of the duration of the stay abroad, the purpose of the visit (business or leisure). Day travels are excluded from the data. The duration of exposure may be smaller for business visits but travellers who fall ill will most likely have returned to their home country and are reported in the Swedish public health system when seeking health care. On the other hand, holiday trips may last one or more weeks. Travellers may be exposed for longer time periods, but when ill may have recovered before returning home. It is difficult to predict in which direction biases may occur but the results suggest that for short-term visits, the risks to travellers may be underestimated. Further biases may be introduced by seasonal travel patterns. It is likely that most travels to the Mediterranean take place in summer, when the prevalence of *Campylobacter* in animals and food is highest. Health-seeking behaviour of travellers or medical decisions about stool cultures may be affected by the country of destination.

A second important assumption is that relative risks to Swedish travellers are predictive of risks for the local population. This assumption ignores any potential effects of acquired immunity, differences in eating habits compared to local residents, and differences between strains circulating in different parts of Europe. It is currently not possible to conclude on the magnitude or even the direction of these biases. A more detailed discussion of potential biases in the data is provided by Ekdahl and Giesecke (2004).

These incidence estimates can be used to update a previous estimate of the EFSA Panel on Biological Hazards (EFSA, 2010d) of the disease burden and costs of campylobacteriosis, and its sequelae. The public health impact is then estimated at 0.35 (90% CI [0.1;0.7]) million disability-adjusted life years (DALYs) per year for the EU-27 with an annual cost of about 2.4 (range 0.9 to 5.1) billion €.

2. Description of the broiler meat production chain

The industrial production of poultry is very diverse. There are two main food production systems: poultry meat (carcasses and processed products), and eggs (for consumption, table eggs; and for further processing, egg products).

Various poultry species, mainly chickens (*Gallus gallus*), turkeys, and guinea fowl, are used in industrial meat production, and their importance varies with regions and food consumption habits.

In 2008, 11.6 millions tons (Tons Equivalent Carcasses) of poultry meat were produced in the EU; comprising mainly broilers (75%), turkeys (16%) and ducks (4%). Poultry meat is the second most important meat species produced in the EU and, at 13% the EU is the third highest poultry meat producer in the world. Nevertheless, since 2007, the importation of poultry meat from third countries is increasing, while export has been slowly decreasing since 2002. In 2008, the amounts of imported and exported poultry meat was quite similar (app. 800 000 tons), indicating the achievement of a self-sufficiency level.

Production of poultry meat and eggs is based on the selection of pure lineages of male and female birds using very precise genetically-influenced criteria, including productivity (growth rate), quality of products and resistance against disease. The selection methods ensure a uniform quality of bird for further multiplication and production. Selection criteria differ according to the type of production. There are also different genetic lines of birds for conventional and free-range or organic production systems.

Most birds are raised in closed ('intensive' or conventional flock) systems. In a conventional flock, the birds are kept inside the houses. Some alternative poultry management systems also exist, such as organic and free-range production. A free-range flock system is a flock production type where the birds have access to the external environment. An organic flock system is a production type that is similar to the free-range system and that fulfils the requirements set for organic production, including birds having access to the external environment and being registered with a recognised Organic Standard Regulatory Organisation and meeting their requirements.

Management systems, which incorporate a free-range phase within the rearing period, vary widely over Europe in terms of period and conditions of free-ranging, and stages prior to free-ranging. The biosecurity measures adopted for the rearing phase, especially in growing units, tend to differ from conventional flocks due to the outdoor access. Much of this variation relates to national management practices. However, for all free-range flocks chicks are placed first in indoor containment before free-range release.

Incursions into the flock occur daily for sanitary and technical reasons, other incursions can occur during the rearing period, including for partial depopulation (thinning), which consists of removing part of the flock before the end of the rearing period.

This procedure has two main objectives:

- Thinning during the rearing period (at 4-5 weeks of age) allows increased weight gain in the remaining birds. The Council Directive 2007/43/EC[9], laying down minimum rules for the protection of chickens kept for meat production, proposes a maximum stocking density in a holding or in a house of a holding lower than 33 kg /m^2 (39 kg/m^2 conditionally). The implementation of this regulation probably encourages the thinning of birds during the rearing period.

- For logistic reasons to optimise slaughter operations, a partial depopulation may be done mainly during the last week of the rearing period. This usually entails the slaughter of the flock over 2 or 3 days.

Before slaughter, feed is often withdrawn from the flock in order to reduce defaecation during transportation, to reduce faecal shedding during defeathering and to facilitate evisceration in the processing plant. Council Directive 2007/43/EC limits feed withdrawal to a maximum of 12 hours before the expected slaughter time.

The slaughter age for broilers varies considerably in the EU. The EU baseline survey (EFSA, 2010a) indicates a minimum of 20 days, a maximum of 150 days, and a mean of 41.4 days. Many producers aim for heavy carcasses and consequently increase the age at slaughter. In organic and some free-range production, the age should not be less than 81 days (Council Regulation (EEC) No 2092/91[10])

The means of transportation of live birds from the farm to the slaughter plant is variable and dependent on the processing company strategy. Currently birds are transported in crates, containers, or cages. Crates and containers are generally introduced into the poultry house and then loaded directly, but cages are loaded outside. The EU Regulation N° 853/2004[11] indicates only some basic requirements concerning the transport of live animals: 1) animals must be handled carefully without causing unnecessary stress; and 2) crates and other equipment must be easy to clean and disinfect, and all equipment must be cleaned, washed and disinfected immediately after emptying and, if necessary, before re-use. Another requirement concerns animals showing symptoms of disease or originating in flocks known to be contaminated with agents of public health importance; these birds may only be transported to the slaughter-house when permitted by the competent authority.

Poultry slaughter and dressing may involve different technologies, dependent mainly on the commercial strategy of the company. In general, birds are placed on the line and stunned using electrical or gas methods, followed by bleeding by neck-cutting. The carcasses are processed using production line speeds of up to 12,000 carcasses per hour. The feathers are first loosened by scalding in warm water, and then mechanically removed by rotating rubber fingers leaving the skin intact. The water temperature in the scalding tank varies between 52˚C and 59˚C depending on whether the end product will be fresh (chilled), or frozen, respectively. Different techniques (soaking, spraying, steam) can be used for scalding carcasses, but soaking seems to be the most effective for loosening the feathers, especially when using a multi-stage facility including counter-current flow to reduce cross-contamination. After removing the head and feet, the viscera are extracted manually or mechanically. The carcasses are then washed in an 'IOBW washer'. After evisceration, carcasses must be chilled to not more than 4°C as soon as possible. This chilling operation can be by immersion in cold water or, most commonly in the EU, in air (dry or with intermittent water sprays). Water chilling is mainly used for frozen products while air chilling is used for refrigerated products.

In smaller abattoirs and local small scale production, manual handling replaces most of the automated processes. Thus, practices like plucking or evisceration may be performed manually.

[9] OJ L 182, 12.7.2007, p. 19–28
[10] OJ L 198, 22.7.1991, p. 1
[11] OJ L 139, 30.4.2004, p. 55–205

Veterinary inspection also occurs during slaughtering and the requirements are described in EU Regulation N° 854/2004 (Annex1, section 1)[12], which lays down specific rules for the organisation of official controls on products of animal origin intended for human consumption. The main task of the official veterinarian is to "verify continuous compliance with food business operators' own procedures concerning any collection, transport, storage, handling, processing and use or disposal of animal by-products for which the food business operator is responsible". For poultry, the ante-mortem inspection can be performed at the holding of provenance or at the slaughter-house, as decided by the competent authority. At the slaughter-house, ante-mortem inspection includes checking the animals' identification (traceability) and screening to ascertain whether animal welfare rules have been complied with and whether signs of any condition, which might adversely affect human or animal health, are present. In the case of clinical symptoms of disease, birds may not be slaughtered for human consumption. In addition, all birds must undergo post-mortem inspection, usually conducted immediately after the evisceration step, although carcasses with obvious abnormalities can be removed even before evisceration in order to minimise cross-contamination. Inspection can be conducted by trained company staff authorised by the competent authority and overseen by an official veterinarian. In particular, the official veterinarian should check that the operators' procedures guarantee, to the extent possible, that meat does not bear faecal or other contamination. If necessary the official veterinarian can instigate further investigations when there is reason to suspect that the meat could be unfit for human consumption. However, visual inspection cannot identify carcasses from *Campylobacter*-colonized birds.

After slaughter, broiler carcasses may be marketed whole or as cuts and portions, or as further processed products, which offer convenience to consumers. As some products and cuts, such as boneless, skin-less breasts, are becoming popular with consumers in certain countries, large factories use automated cutting and processing equipment, while smaller operations employ manual cutting procedures. Cutting of broiler carcasses involves removal of legs, wings and the breast, while the legs and leg quarters may be further cut into thighs and drumsticks. The wings can also be sold as such, or cut into drumettes, while the remaining carcass racks are used for production of soup stock, pet food, or waste. Portioning and sizing are needed for situations where uniform portions of meat are served. In addition to deboning, cutting, portioning and packaging, further processing involves operations such as tumbling, massaging, reforming, emulsifying, breading or battering, marinating, and partial or complete cooking. Forming is done by reducing the size of meat cuts, mixing with brines containing binding and flavouring ingredients, tumbling to increase penetration of brine, and forming or moulding into appropriate sizes and shapes with extruder/stuffers. In general, further processing of broiler carcasses may be as simple as splitting the carcass into halves or as complex as producing marinated or breaded, partially or fully cooked products (Mead, 2000).

3. Baseline study on *Campylobacter* in the broiler meat chain in the EU

An EU-wide baseline survey on *Campylobacter* in broiler batches and on broiler carcasses was carried out in 2008 (EFSA, 2010a). In this survey a total of 10,132 broiler batches were sampled from 561 slaughter-houses. From every batch (assumed to represent a group of chickens raised together in one shed) one pooled sample from the caecal contents of 10 carcasses was examined for *Campylobacter*. From the same batch, one carcass was collected after chilling. From this carcass the neck skin and breast skin were examined for the presence of *Campylobacter*. In addition, *Campylobacter* counts were determined. The results are as follows:

3.1. *Campylobacter* in broiler batches

The EU baseline survey showed that at the Community level the prevalence of *Campylobacter*-colonization in broiler batches, as determined from caecal contents was 71%. The prevalence of positive batches varied widely between EU Member States from 2.0% to 100.0% (EFSA, 2010a). One consistent feature throughout the literature and confirmed in the EU baseline survey, is that northern European countries (above 57° latitude; notably Sweden, Norway, Finland and Denmark) had lower

[12] OJ L 139, 30.4.2004, p. 206–320

prevalences than more southern MS. However, there is no clear delineation based on latitude. From 50˚ to 55˚ Belgium and the Netherlands have a relatively low prevalence while the UK, Ireland, Poland and Germany have a relatively high prevalence. Six out of the 12 countries with the highest prevalences over, at or above the EU median, are wholly or partly located below the 45˚ latitude. This trend suggests that latitude influences the prevalence of *Campylobacter* colonization in national flocks. However, clearly multiple factors are involved in these observations. These may include weather patterns, seasonality of colonization, and differences in industry structure and management. For example, climatic conditions, may affect the reservoirs or vectors of *Campylobacter* in the environment (Jore *et al.*, 2010; Meremaee *et al.*, 2010). Moreover, most northern European countries have actively implemented successful strategies to control *Campylobacter* and have consequently achieved a reduction in the prevalence of *Campylobacter* in broiler flocks (Rosenquist *et al.*, 2009).

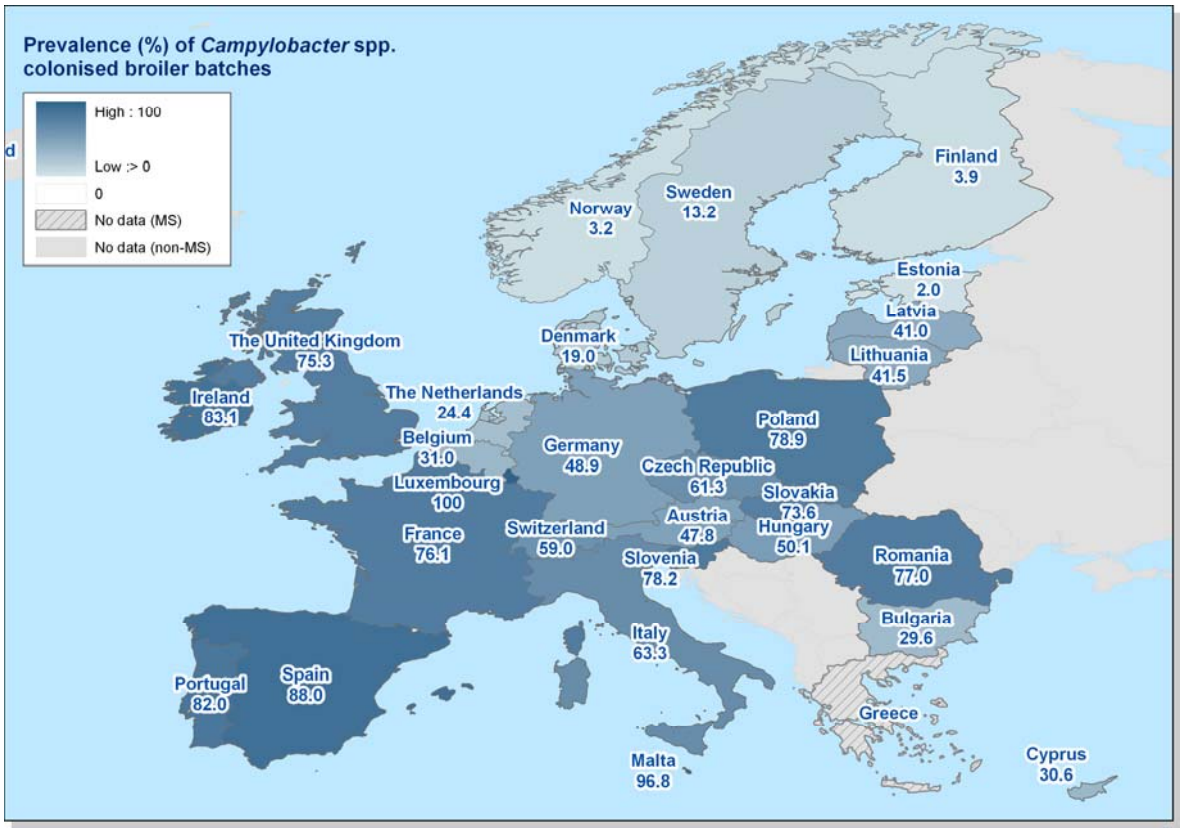


Figure 2: Prevalence of *Campylobacter*-colonized broiler batches in the EU, 2008 (EFSA, 2010a)

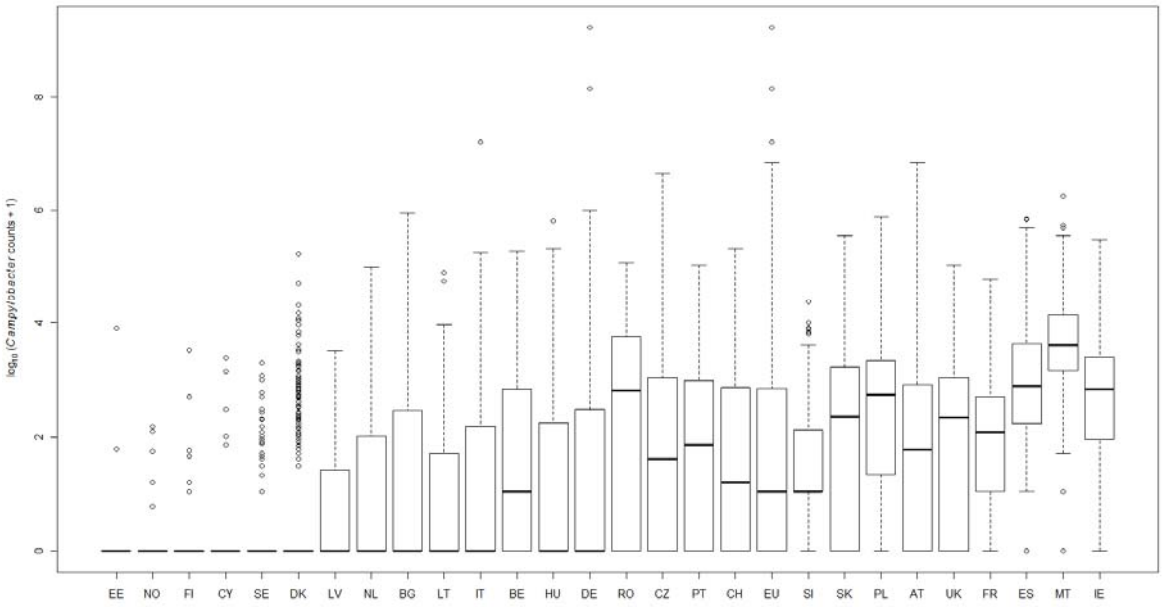
Many countries demonstrate a seasonal peak in flock prevalence in mid-summer. The shape and timing of this peak varies, with the northern European countries having much sharper summer peaks in prevalence compared to the more southern countries (EFSA, 2010a; Nylen *et al.*, 2002). Many reasons for these national differences in the summer peak have been suggested including differences in the presence of flies, higher water run-off, increased ventilation needs related to temperatures, etc. It is likely that the causes of the summer peak vary between regions, i.e. flies in one region but contaminated water run-off in another. With climate change, the summer peak may become even more important in the future.

3.2. *Campylobacter* on broiler carcasses

In the EU baseline survey one carcass was collected after chilling from the same batch as the one that was sampled for ceacal contents. The neck and breast skin were also examined for presence of and numbers of *Campylobacter*. At the Community level the prevalence of *Campylobacter*-contaminated broiler carcasses was 75.8%. The MS-specific prevalences (based upon any detected *Campylobacter*

by either the detection method or the enumeration method) varied markedly. MS prevalence ranged from a minimum of 4.9% to a maximum of 100.0%.

The results of the counts of *Campylobacter* on broiler carcasses showed substantial variation in contamination levels among the MSs. The proportion of samples considered negative by the enumeration test (i.e. below the threshold of 10 CFU/g) varied from 3.8% to 98.6% among MSs, whereas the proportion of samples with very high counts, (i.e. above 10,000 CFU/g) varied from 0% to 31.9% (EFSA, 2010a).



Note: In the boxplots, the bottom of the box represents the first quartile of the distribution and the top of the box the third quartile, whereas the bar inside the box represents the median. Small circular symbols indicate extreme values, differing from the box > 1.5 times the difference between the third and the first quartile (interquartile range).
* No *Campylobacter* enumeration was executed in broiler carcass samples from Luxembourg; Greece did not participate in the baseline survey; and two non-MSs, Norway and Switzerland, participated. *Campylobacter* counts were added to one previous to $\log_{10}$ transformation in order to allow for the inclusion of negative counts (< 10 CFU/g) in these representations.

Figure 3: Boxplot of the $\log_{10}$ (*Campylobacter* counts on broiler carcasses + 1), by country and in the EU*, 2008 (EFSA, 2010a)

When comparing MS-specific figures for the prevalence of *Campylobacter*-colonized broiler batches and *Campylobacter*-contaminated broiler carcasses with *Campylobacter* enumeration results, a tendency is observed for countries having a higher *Campylobacter* prevalence in both slaughter batches and carcasses, to have higher quantitative loads on carcasses.

4. *Campylobacter* in the broiler meat production chain

The accrued evidence and expert opinion from the last 25 years indicates that control of *Campylobacter* in the broiler meat production chain should reduce human campylobacteriosis (Tustin *et al.*, 2011). Any strategy to control *Campylobacter* in broiler meat should be based on the stringent application of good hygienic practices (GHP) through all stages of the food chain, i.e. in primary production; during transportation and before slaughter; and at slaughter, dressing and processing; as well as during retail and preparation in professional or private kitchens. In this Opinion, the focus is on the first three stages of the food chain as these are most relevant for legislation at the EU level. However, national control strategies should take all stages into account.

Although broiler meat is an important source of exposure, *Campylobacter* originating from chickens and dispersed into the environment may also be important (see also Chapter 1). This suggests that

controlling *Campylobacter* on the broiler farm will have more impact on public health than control later in the food chain, even though the relative impact of different pathways cannot currently be quantified more precisely. Based on these considerations and on general principles of food hygiene, a hierarchy of control methods has been considered:

- Prevent *Campylobacter* entering broiler houses at primary production (using approaches primarily based on biosecurity including hygienic measures during thinning, and possibly by reducing slaughter age);
- Increase resistance of broiler chickens to colonization (using approaches such as additives (organic acids, phytocompounds) to drinking water and/or feed, vaccination, and/or selective breeding);
- Reduce the concentration of *Campylobacter* in chicken intestines before slaughtering (for example by treatment with bacteriophages or bacteriocins)
- Enhance hygienic measures during slaughter (for example improved design of equipment, slaughter practices, prevention of faecal leakage, training of personnel in hygiene implementation,
- Apply decontamination of carcasses (using chemical or physical treatments); and
- Educate food handlers in hygienic practices (professional catering and domestic household setting, primarily aimed at prevention of cross-contamination during handling of broiler meat).

This chapter aims to provide a quantitative estimation of the impact of a wide range of interventions on *Campylobacter* at relevant stages in the food chain and possible advantages and disadvantages as well as current availability. Where relevant, for selected interventions, the data have been used to inform CAMO (see Chapter 5). Interventions currently in the early stages of development have been included, as well as those which have been under development for a longer time and for which more data are available. The inclusion of, as yet untried, novel and innovative interventions, was considered important in order to guide further research and development and to assist in the development of a longer-term strategy. Risk managers may base their decisions on the suggested hierarchy of control methods in combination with their effectiveness and availability, taking local conditions into account.

4.1. Risk factors for *Campylobacter* in primary production

As *Campylobacter* can persist and multiply in many hosts and can survive in some environments, there are multiple potential sources and routes of flock infection. Identification of the on-farm risk factors for the introduction of *Campylobacter* into flocks has proven very difficult. The major research approach adopted has used risk factor-based farm surveys, involving structured questionnaires. Most risk factor studies have involved cross-sectional surveys with sampling later in the production chain (i.e., at or just before slaughter). In recognition of the deficiencies of such sampling regimes, more recent studies have adopted a longitudinal approach with sequential sampling (usually weekly) throughout the growing period. When supplemented with culturing of environmental samples (e.g. water, poultry houses, etc), longitudinal sampling has been used to indicate environmental sources. Most recently, molecular epidemiology has also been applied to compare the isolates from the environment with those from the chickens. By combining longitudinal sampling with molecular strain typing, the direction of transmission may be detectable, i.e. from the environmental source to the birds or vice versa.

Surveillance studies have shown that there are multiple risk factors associated with *Campylobacter* infection in broilers (Kapperud *et al.*, 1993; Lyngstad *et al.*, 2008; Newell and Fearnley, 2003). Some risk factors are frequently implicated regardless of the country investigated or the robustness of the study design. These primary factors include season, increasing bird age, poor biosecurity (including lack of house-specific clothing), thinning (partial depopulation), the presence of flocks of various ages

on the farm, the farming of multiple species, and use of extensive rearing at any stage. Other risk factors are more intermittently implicated. These include the use of non-potable drinking water, lack of farmer awareness regarding the importance of biosecurity, the presence of insects or vermin and, the use of antibiotics. These secondary risk factors may be related more to specific management practices or even geographical location. Published information on risk factors for *Campylobacter*-positive flocks have been reviewed by Adkin *et al.* (2006). Despite the many published studies, the relative importance of each of these risk factors for flock positivity remains unknown, but will probably vary from farm to farm. A systematic review of the literature (Newell *et al.*, 2008) indicates that poor study designs, implementation, and analysis of risk factor have contributed substantially to this lack of knowledge.

4.1.1. Vertical transmission

Vertical transmission of a pathogen in poultry is usually defined as the internal contamination of the egg within the genital tract and before intact shell deposition. The evidence on vertical transmission indicates that the congenital acquisition of *Campylobacter* infection occurs rarely. Although *Campylobacter* can be recovered from the hen oviduct (Camarda *et al.*, 2000), the prevalence of *Campylobacter* inside commercial eggs is very low (Shanker *et al.*, 1986), or non-existent.

However, it can also be more loosely interpreted to include the transmission of organisms from parent flocks to progeny via routes such as faecal contamination of shells. Although transmission from breeder to progeny flocks from contaminated hatchery material is possible, there is no epidemiological confirmation of this, which probably reflects the poor survival of organisms on eggs during incubation, hatching and transportation to the farm.

Attempts have also been made to isolate live bacteria from hatchery fluff, debris and/or liners (Byrd *et al.*, 2007; Callicott *et al.*, 2006; Jacobs-Reitsma *et al.*, 1995; Kiess *et al.*, 2007). Most studies were unable to recover cells and even PCR failed to detect DNA (Callicott *et al.*, 2006). However, in one report (Byrd *et al.*, 2007) 0.75% of samples were culture-positive. Thus, fertile eggs and hatchery debris entering the farm environment may be contaminated with viable organisms. Nevertheless, the role of such contamination in flock colonization is debatable for the following reasons:

- the consistent lag phase observed in commercial flocks and the failure to isolate *Campylobacter* from birds during this period (Evans and Sayers, 2000; Kiess *et al.*, 2007);
- the absence of colonization in the thousands of chicks hatched under laboratory conditions and maintained under high level biosecurity to supply control groups for experimental colonization studies (Newell and Fearnley, 2003);
- the absence of infection in progeny chicks placed under experimental high biosecurity containment compared with siblings remaining in commercial conditions (Smith *et al.*, 2004); and
- the comparative molecular typing of isolates from parent and progeny flocks (Jacobs-Reitsma, 1995; Pearson *et al.*, 1993; Petersen *et al.*, 2001b).

As chicks are considered free of *Campylobacter* on the day of hatching (Newell and Fearnley, 2003), each broiler cycle is considered to start with a *Campylobacter*-negative flock. Whether the flock becomes colonized with *Campylobacter* is then dependent on how effective the biosecurity measures are at excluding *Campylobacter* from the broilers.

In the recent EU baseline survey of *Campylobacter* in broiler batches (EFSA, 2010b), multivariable regression analysis showed a number of significant risk factors for *Campylobacter*-positive batches, including partial depopulation, slaughter age, and season. Interestingly, additional analyses performed for two MSs-groups, notably countries with high and low prevalences of *Campylobacter*-colonized

broiler batches, using the median prevalence (54.6%) as the cut off point, identified the same potential risk factors, i.e. thinning, age, and season, as significant in both groups.

Overall vertical transmission does not appear to be an important risk factor for colonization of broiler chickens with *Campylobacter* and preventing vertical transmission is generally not considered as an intervention.

4.1.2. Slaughter age

The analysis of the pooled caecal contents from the EU baseline survey (EFSA, 2010b) indicated that the age of the birds at slaughter was a risk factor in terms of increasing prevalence of *Campylobacter* colonization of broiler batches per 10 days of age. Age has previously been identified as a risk factor for *Campylobacter* colonization in broilers at slaughter in several countries (Bouwknegt *et al.*, 2004; Evans and Sayers, 2000; Hartnett *et al.*, 2001).

Most conventionally reared flocks are *Campylobacter*-negative for the first three weeks of life. Thereafter, the prevalence of colonized flocks increases. Infection once acquired is sustained in most broiler flocks until slaughter; however, in flocks of birds older than 8-9 weeks (i.e., organic, free-range or laying hens) the proportion of positive birds in a flock may decline, possibly as a result of acquired immunity. As the age of slaughter can vary substantially between countries and flock types this could contribute to variations in flock prevalence throughout the EU.

There are several explanations for the increased risk of colonization with age, including increased risk of exposure to infection and changing susceptibility of the birds. It is reasonable to assume that the chance of contamination entering a broiler house increases over time, i.e. with increasing age of the birds. This reflects the increasing number of challenges to the biosecurity barrier by farm staff and the increasing volumes of water, feed and air required as birds became bigger.

4.1.3. Season

From the EU baseline survey (EFSA, 2010b), season (quarter of sampling during the year) appeared to be a risk factor (OR= 4.07, CI [3.09;5.36] for the period July-September compared to January-March) for *Campylobacter* colonization of broiler batches.

Seasonal variation in *Campylobacter* prevalence in broilers, with a peak in the summer has been previously reported from several countries in northern Europe, e.g. Sweden (Hansson *et al.*, 2007a), Denmark (Wedderkopp *et al.*, 2000), Norway (Hofshagen and Kruse, 2005), and the Netherlands (Bouwknegt *et al.*, 2004). These observations are supported by the EU baseline survey (EFSA, 2010b). Similar observations have also been reported from regions of France (Refregier-Petton *et al.*, 2001) but were not supported by the data from France in the EU baseline survey. Other national studies in the United Kingdom, USA, and Canada have previously reported no seasonal influence on *Campylobacter* prevalence (Gregory *et al.*, 1997; Humphrey *et al.*, 1993; Nadeau *et al.*, 2003). The EU baseline survey (EFSA, 2010b) clearly demonstrates that, at least in some cases, this difference is a reflection of the ratio of prevalence of colonization between summer and winter months. With a high prevalence in winter, a summer peak would not be so readily observed. The more northern countries tend to have few, if any, colonized flocks during October-March.

The reason for the association of higher *Campylobacter* prevalence and season is not known, but appears to be temperature-related (Jore *et al.*, 2010). It has been suggested that the seasonality could be related to the abundance of flies in the summer which act as as mechanical vectors (Hald *et al.*, 2008). During summer, ventilation and water consumption also increase because of the higher temperatures.

The fact that low winter prevalence appears important to seasonality, seems to indicate differences in the importance of risk factors at various times of the year, and in different countries. One possible explanation is that the colder winters of northern Europe reduce the environmental *Campylobacter* burden around poultry farms, thus reducing the risk of colonization. However, this too is not clear cut

as Canada, which has severe winter conditions, also has a high national prevalence during the winter months (Nadeau *et al.*, 2003).

4.1.4. Thinning

Some farmers partially depopulate or "thin" flocks (see Section 2). The process of thinning entails many people and much equipment inside the poultry house for a considerable time, and if the personnel or equipment are contaminated with *Campylobacter*, the chance of transmitting *Campylobacter* to the house environment and to the flock may be substantial, depending on the hygienic measures taken.

Several surveys have found a statistically significant risk associated with thinning (Allen *et al.*, 2008b; Bouwknegt *et al.*, 2004; Hald *et al.*, 2001; Puterflam *et al.*, 2005; Refregier-Petton *et al.*, 2001; Wedderkopp *et al.*, 2000), and that the risk is greatest when the thinning crews are large (Puterflam *et al.*, 2005), visit more than one farm, or are poorly educated (Berndtson *et al.*, 1996). The impact of thinning in some EU countries, such as the UK, is generally considered high (Evans, 1992). However, one study in the Netherlands (Russa *et al.*, 2005), based on 1,737 flocks, questioned the importance of thinning as a risk factor. Similarly, the CARMA project showed no big impact of thinning on prevalence (Havelaar *et al.*, 2007). Thus, the effects of thinning are debatable.

One factor possibly influencing these somewhat contradictory results is the variable time (between one and seven days) between the thinning and final depopulation. If this time is short, then any *Campylobacter* introduced into the house during thinning may not have spread sufficiently throughout the flock to be detectable before final slaughter. A possible confounding factor is the effect of increased age of the remaining birds in the house before the next depopulation, as age is an independent risk factor as discussed in Chapter 4.1.1. The stress that birds experience during thinning might assist the establishment and spread of *Campylobacter* through the flock (Cogan *et al.*, 2007). Such stresses would be expected to increase neurotransmitters such as noradrenaline which are said to enhance subsequent colonization by *Campylobacter* (Humphrey, 2006).

4.1.5. Farm staff and farm visitors

It is widely assumed that farm workers and visitors to the farm, such as bird catching crews and maintenance personnel, constitute risk factors for colonization of the flock, and that human traffic is an important vehicle for *Campylobacter* being introduced into the poultry house from the external environment (Berndtson *et al.*, 1996; Cardinale *et al.*, 2004; Evans and Sayers, 2000; Hald *et al.*, 2000; Kapperud *et al.*, 1993). This seems logical given that the boots of such workers can be *Campylobacter*-positive (Herman *et al.*, 2003; Ramabu *et al.*, 2004) and molecular epidemiology provides evidence that these strains subsequently colonize target flocks (Messens *et al.*, 2009; Ridley *et al.*, 2008a). In many cases catching crews are based at poultry processing plants and, like maintenance personnel, travel from farm-to-farm with their own equipment, boots and clothing, frequently without due regard to personal hygiene or biosecurity issues. However, when crews are trained and dedicated to one farm the risk may be small (Berndtson *et al.*, 1996; Romero Barrios *et al.*, 2006). Personnel handling other animals, especially other poultry local to the target house, increase the risk. In addition, both the number of staff allocated to the target house and the number of visits to the target house per day are directly related to the risk.

4.1.6. *Campylobacter*-contaminated drinking water and feed

Campylobacter can survive in water for weeks and persist in run-off from pasture and in waste water from a variety of sources, such as slaughter plants and even sewage plants, eventually getting into the water-table, surface waters or water reservoirs. Thus, water treatment failure, use of unchlorinated water sources from public suppliers or from wells, or contamination of on-farm water reservoirs may enable the delivery of live *Campylobacter* to poultry houses. Water lines can also become contaminated either from *Campylobacter* in water entering the house or from the back-tracking of organisms from drinkers contaminated by positive birds. Water in drinkers can also be contaminated

and act as a rapid vector of *Campylobacter* around the flock. Nipple drinkers with drip cups are more frequently contaminated than those without (Cox and Pavic, 2009).

Water source or infrequent sanitation or disinfection of water lines are reported as significant risks in some (Arsenault *et al.*, 2007; Berndtson *et al.*, 1996; Evans and Sayers, 2000; Gibbens *et al.*, 2001; Herman *et al.*, 2003; Lyngstad *et al.*, 2008) but not all, risk factor studies (Guerin *et al.*, 2007; Hald *et al.*, 2000; McDowell *et al.*, 2007; Neubauer *et al.*, 2005; Refregier-Petton *et al.*, 2001; Rosef *et al.*, 2001). On the other hand, Refrégier-Petton (2001) surprisingly found acidification of drinking water as a risk factor for contamination of broiler flocks with *Campylobacter*. The authors explained this by indicating that acidification might be an indicator of the poor sanitary status of drinking water given to the birds. Although attempts to isolate *Campylobacter* from water supplies before flock infection have been unsuccessful (Gregory *et al.*, 1997; Hansson *et al.*, 2007b; Hiett *et al.*, 2002; Kapperud *et al.*, 1993; Lindblom *et al.*, 1986; Pearson *et al.*, 1993; Ring *et al.*, 2005), *Campylobacter* is notoriously difficult to isolate from water, even when detectable by immunofluorescence (Pearson *et al.*, 1993). Recent evidence suggests that biofilms (Kalmakoff et al, 2006; Zimmer *et al.*, 2002) and protozoa (Axelsson-Olsson *et al.*, 2005; Bare *et al.*; Snelling *et al.*, 2008) in the drinking water system are of potential importance.

Mills and Philipps (2003) failed to find *Campylobacter* in feed. In vitro, evidence indicates that lack of moisture renders feed a hostile environment for *Campylobacter*.

4.1.7. Insects

The ambient body temperature of insects is unlikely to support *Campylobacter* multiplication. However, insects, such as houseflies, are attracted to and feed off faeces from livestock and other animals, fly to new locations and then passively transfer the faecal material at the next location, which might be a poultry house. In contrast, many insects, such as darkling beetles, are permanent residents within poultry houses, live in protected environments deep within the walls and may passively carry faecal contaminants from previous flocks. It seems likely that the insect populations in and around poultry houses vary with location, season and management practices thus, generic statements about the role of insects may not be possible. Nevertheless, up to 20% of the external surfaces and 70% of the viscera of exposed houseflies can be contaminated with *Campylobacter* (Shane *et al.*, 1985). However, the presence of insects, including darkling beetles, or the use of insecticide were not significant factors in risk factor surveys in Sweden (Berndtson *et al.*, 1996) or France (Refregier-Petton *et al.*, 2001). In on-farm studies, *Campylobacter* were either not cultured from insects (Neubauer *et al.*, 2005) or were only recovered after the flock became positive (Bates *et al.*, 2004; Jacobs-Reitsma *et al.*, 1995). In contrast, in a Danish study (Hald *et al.*, 2004), 8.2% of 49 flies captured from around a poultry house were culture-positive for *Campylobacter* while 70.2% of 47 flies were PCR positive. A further study (Hald *et al.*, 2008) estimated that 30,000 flies entered a house during one rearing cycle, thereby constituting a high risk of transmission to poultry. However, in those countries with a high winter prevalence, the temperatures in winter would be too low to sustain fly populations and therefore the role of flies as a source in winter would be questionable.

4.1.8. Wild animals (including rodents) and birds

Lack of vermin control is also a risk factor (Arsenault *et al.*, 2007; Huneau-Salaun *et al.*, 2007). *Campylobacter* is readily recovered from the faeces of most wild animals and wild birds. However, the evidence for these as sources of on-farm contamination is sparse and contradictory. Some subtypes (by *fla*A SVR or AFLP), isolated from wild bird faeces in the farm, were later found in broiler flocks (Hiett *et al.*, 2002; Johnsen *et al.*, 2006). However, this has not been confirmed in other studies (Colles *et al.*, 2008; Petersen *et al.*, 2001a). The evidence for rodents as on-farm sources is also circumstantial. The presence of rodents on farm can have a strong association with flock positivity (McDowell *et al.*, 2007; 2008), while in another study rodent contamination was identified before chicken positivity (Gregory *et al.*, 1997).

4.1.9. Livestock on farm or within the locality

The carriage rate of *Campylobacter* in the gastrointestinal tract of livestock is high. In a national baseline survey of cattle, sheep and pigs, the carriage rates were 54.6%, 43.5% and 69.3%, respectively, in Great Britain (Milnes *et al.*, 2008). The evidence from the few studies undertaken indicates that the risk to poultry flocks from positive livestock in the same locality is high (Cardinale *et al.*, 2004; van de Giessen *et al.*, 1996). In a Norwegian case control study on broiler farms (Lyngstad *et al.*, 2008) using a multivariate logistic regression analysis with *Campylobacter* positivity as the outcome, the presence of a swine farm closer than two kilometres had an Odds Ratio of 1.6 (95% CI [0.7; 3.2]).

Molecular epidemiological approaches indicates that strains colonizing poultry flocks may sometimes be found in adjacent livestock, including cattle and pigs, (Jacobs-Reitsma *et al.*, 1995; Johnsen *et al.*, 2006; Ridley *et al.*, 2008a), and in longitudinal studies this can be detected prior to poultry flock colonization indicating that the direction of transmission is from the livestock to the broilers. However, strains in livestock adjacent to the broilers are not always subsequently recovered from the broilers (Ridley *et al.*, 2011).

4.1.10. Presence of *Campylobacter* in the farm environment

Once a flock becomes positive, then the surrounding farm environment becomes widely contaminated (Herman *et al.*, 2003) and contamination can persist for several weeks (Johnsen *et al.*, 2006). However, *Campylobacter* is also widespread in the environment around poultry houses (Hansson *et al.*, 2007b) even before bird placement. Standing water or puddles are particular sites from which *Campylobacter* can be recovered. There is a close association between such environmental contamination and the weather (Hansson *et al.*, 2007b), and recovery is highest just after rain. Frequently the genotypes of isolates from puddles and house surroundings are identical to those later isolated from the flock when it becomes positive (Hansson *et al.*, 2007b; Hiett *et al.*, 2002; Messens *et al.*, 2009; Rivoal *et al.*, 2005), indicating that the "tracking-in" of these strains is an important source of infection for the flock.

Surveys of free-range flocks (organic or not) generally indicate that such flocks have a higher prevalence of colonization than conventionally-reared flocks (McCrea *et al.*, 2006; Ring *et al.*, 2005). In longitudinal studies of free-range flocks (Colles *et al.*, 2008; Huneau-Salaun *et al.*, 2007), colonization with *Campylobacter* is mainly concurrent with release to free-range. Thus, the general assumption is that exposure to the free-range environment causes positivity due to exposure to environmental contamination. However, this assumption has recently been questioned (Colles *et al.*, 2008) on the basis of lack of commonality, by molecular typing, between strains from wild birds in the environment of the free-range farm. Certainly some of the flocks are positive before they are released onto pasture and multiple sources and routes of transmission are suspected (Huneau-Salaun *et al.*, 2007), including wild birds, other livestock etc, based on molecular epidemiology. Free-range birds are also older when slaughtered than conventionally-reared birds, which may be reflected in the prevalence.

4.1.11. A previous *Campylobacter*-positive flock in the house (carry-over)

One potential source of *Campylobacter* is a previous positive flock in the same house. *Campylobacter* from a positive flock becomes widely disseminated in and around the house (Hiett *et al.*, 2002). Thus, the risk of carry-over of infection should be directly related to the efficiency of cleaning and disinfection as well as down-time between flocks.

Campylobacter recovery from the inside of a poultry house after cleaning and disinfection and before or during placement of chicks has not been reported (Cardinale *et al.*, 2004; Evans and Sayers, 2000; Herman *et al.*, 2003; van de Giessen *et al.*, 1992). This is consistent with multiple longitudinal studies undertaken with sequential flocks which report that carry-over of *Campylobacter* infection from one flock to a subsequent one in the same house is limited (Colles *et al.*, 2008; Evans and Sayers, 2000;

Jacobs-Reitsma *et al.*, 1995; McDowell *et al.*, 2008; Shreeve *et al.*, 2002) and that colonization can occur just as readily in new houses as in previously used houses (Gregory *et al.*, 1997).

Molecular epidemiological investigations of carry-over have been particularly useful because they can use highly discriminatory techniques to identify particular strains in sequential flocks in target houses. In a major study (Shreeve *et al.*, 2002) of strains from 100 sequential flocks in Great Britain typed by *fla*A-typing, only 16% had evidence of possible house-specific strain carry-over from a previous positive flock in the same house. Similar observations were made in the Netherlands (Jacobs-Reitsma *et al.*, 1995). These data suggest that carry-over appears limited, although it could still account for 10-20% of infections in new flocks, and the inadequate disinfection of the poultry house and its environment are therefore important risk factors.

The time between flocks (the downtime), provides an opportunity for loss of *Campylobacter* viability and therefore longer downtimes could reduce the carry-over of infection. The results of risk factor analysis to date have been variable (Berndtson *et al.*, 1996; Lyngstad *et al.*, 2008). Anecdotally downtimes can be as long as 25 days or as short as 3 days.

4.1.12. Use of therapeutic antimicrobials for treatment

As *Campylobacter* colonization is asymptomatic in chickens there is no commercial benefit for the therapeutic treatment of flocks to eliminate the colonization. However, the treatment of flocks with certain antibiotics, for health reasons such as bacterial respiratory infections, during rearing, can affect the dynamics of colonization in birds already colonized by *Campylobacter* (Stapleton *et al.*, 2010). Although Herman *et al.* (2003) found no effect on the excretion of *Campylobacter,* Refregier-Petton *et al.* (2001), looking at risk factors, concluded that the administration of an antibiotic decreased the risk of a flock being colonized by *Campylobacter* (OR=0.1, 90% CI [0.1;0.6]).

Importantly such therapeutic treatment may induce antibiotic resistance in antibiotic-sensitive bacteria already colonizing the birds. This is particularly seen as a risk for fluoroquinolone resistant *Campylobacter* (Geenen *et al.*, 2011), and the use of antimicrobials to control *Campylobacter* in broilers is strongly discouraged.

4.1.13. Unused or used litter and waste disposal on site

Campylobacter was not detected in unused litter (Jacobs-Reitsma *et al.*, 1995) probably because the lack of moisture renders it a very hostile environment for the organism. Thus fresh litter is an unlikely source of contamination in the house.

Campylobacter survival in poultry litter has been determined to have a D-value (length of time for one $\log_{10}$ decrease) of 2.53 days (Hutchison *et al.*, 2005). When litter becomes wet this might enhance survival and increase the risk of infection up to two-fold (Berndtson *et al.*, 1996). If the distance between the stacked used litter and the target house is less than 200 metres then the risk of flock colonization may increase five-fold or more (Arsenault *et al.*, 2007; Cardinale *et al.*, 2004) presumably because of seepage or run-off. In addition, the presence of dead birds on the farm increases the risk of positive flocks (Evans and Sayers, 2000).

The type of litter used appears to have no effect on the risk of flock infection (Neubauer *et al.*, 2005). Reused litter (which is a regular occurrence in the USA but not Europe) may provide some protection (Kiess *et al.*, 2007), at least in turkeys, possibly as a result of enhancing the diversity of the gut flora.

4.1.14. *Campylobacter*-contaminated air

Air sampling studies indicate that air becomes contaminated only after the flock in the house has been colonized (Pearson *et al.*, 1993). Nevertheless, both the construction and dynamics of ventilation systems can influence the risk of flock positivity (Refregier-Petton *et al.*, 2001; Romero Barrios *et al.*, 2006).

4.1.15. Stocking density and flock size

The evidence for an association between flock positivity and stocking density (Kapperud *et al.*, 1993; Romero Barrios *et al.*, 2006) or flock size (Berndtson *et al.*, 1996; Evans and Sayers, 2000; Kapperud *et al.*, 1993; Romero Barrios *et al.*, 2006) is contradictory, and therefore no conclusion can be made.

4.1.16. Number of houses on site

The risk of *Campylobacter* colonization in a target poultry house increases with the number of houses on the site; however, any association between degree of risk and the number of houses on a farm is unclear. A Norwegian study showed an increased risk of having a *Campylobacter* positive flock if the farm had more than one house (OR= 3.7, CI [1.0;14.5]) (Lyngstad *et al.*, 2008). With every additional house there is an increased number of biosecurity barrier passes and possibly an increase in farm staff. More importantly the first house that becomes positive provides a massive reservoir of infection via cross-contamination for all the remaining houses.

4.1.17. Bird health

Campylobacter is recognised as a commensal in chickens and there is no recorded effect of *Campylobacter* colonization on bird weight gain or feed conversion. Risk factor surveys have specifically reported (Berndtson *et al.*, 1996; Hald *et al.*, 2000; Kapperud *et al.*, 1993) that there is no significant association between bird health and *Campylobacter* flock prevalence. However, an association between *Campylobacter* flock positivity and bird health has been suggested (Humphrey, 2006) and has been supported by some epidemiological evidence (Bull *et al.*, 2008).

4.2. Interventions against *Campylobacter* in primary production

At primary production a multi-layered intervention strategy would be optimal with sequential intervention approaches targeting different events in the infection cycle. The primary approach would be to prevent *Campylobacter* entering the flock (primarily by biosecurity). If that fails, then reducing flock susceptibility to infection would be the next step (e.g. by feed and water additives, vaccination, or selective breeding). Both control options should reduce the numbers of poultry-associated *Campylobacter* in the environment as well as those entering the food chain. If both these approaches fail to prevent infection then the next step would be to reduce the number of *Campylobacter* in the bird gut at the time of harvest (e.g. by dosing with bacteriophages or bacteriocins). Post-harvest measures, such as improved processing and decontamination, would then need to deal with any remaining *Campylobacter*-positive carcasses at the abattoir level.

4.2.1. Biosecurity

Biosecurity is a set of preventative measures implemented to reduce the risk of transmission of infectious disease from reservoirs of the infectious agent to the target host. Clearly security plans will need to vary depending on the circumstances; some risks will be generic and global while others will depend on time, location, resource availability, etc.

A conventional poultry house, that is modern and well maintained and with limited access, should be considered biosecure. If biosecurity is strictly and consistently implemented then no *Campylobacter* (or other pathogenic agents) is transported from outside the house to the inside. However, in practice it is very difficult to obtain and maintain such a level of biosecurity. Passive transgressions of the biosecurity perimeter in such a house may be through essential commodities like water, feed, and air. Active transgressions require the carriage of *Campylobacter* from the external environment, which may occur by vectors such as vermin or flying/crawling insects, but the most visible vehicles are humans. Once *Campylobacter* enters the broiler house and infects the first birds, spread is very rapid and virtually all birds are colonized within one week. Hence, the aim of biosecurity measures is primarily to control the entry of *Campylobacter* into the house.

Biosecurity in free-ranging flocks requires a different approach. Enclosure, enabling restricted access by wildlife, insects etc, may only occur for part of a flock's life. Nevertheless, some components of generic biosecurity, such as clean water and feed, changing boots and washing hands, vaccination etc, can still be enforced and will provide some degree of protection. This level of biosecurity is, however, unlikely to control *Campylobacter*, which explains the higher prevalence of flock colonization in free-ranging and organic flocks.

4.2.1.1. Hygiene barriers

Hygiene barriers at the entrance to poultry flocks are a very important part of the biosecurity measures on a farm, but may vary in location, structure and, most importantly, use. The purpose of a hygiene barrier should be to physically separate the "dirty" outside environment from the "clean and protected" inside environment.

In the most basic situations a hygiene barrier for a poultry house is a physical point (possibly as simple as a line drawn on the floor serving as a passive barrier) at which there is a boot dip and/or a change of footwear. Hygiene barriers may also enable/ensure a change of outer clothes (overalls), and provide hand washing facilities. The strict use of a hygiene barrier may reduce the risk of flock infection by about 50% (Berndtson *et al.*, 1996; Evans and Sayers, 2000; van de Giessen *et al.*, 1998), and seems especially important when there are other livestock on the farm (Hald *et al.*, 2000; van de Giessen *et al.*, 1992). All intervention studies undertaken have included hygiene barriers (Gibbens *et al.*, 2001; van de Giessen *et al.*, 1998).

The use of house-specific boots (Bouwknegt *et al.*, 2004; Evans and Sayers, 2000; van de Giessen *et al.*, 1996) and clothes (Bouwknegt *et al.*, 2004; Hald *et al.*, 2000), overshoes (Puterflam *et al.*, 2005) and effective use of boot dips (Bouwknegt *et al.*, 2004; Evans and Sayers, 2000; Gibbens *et al.*, 2001; McDowell *et al.*, 2007; 2008; van de Giessen *et al.*, 1996) are all associated with a reduced risk of flock infection. However, the benefits of house-specific boots compared to boot-dips remain unclear. Intervention studies (van de Giessen *et al.*, 1992; 1998) provide no indication of the reduction in risk associated with the use of separate boots.

As far as it is known, there has been only one controlled intervention trial undertaken (in the UK) to assess whether the risk of a broiler flock becoming colonized by *Campylobacter* could be reduced by defined biosecurity measures (Gibbens *et al.*, 2001). In this study, farmers managing intervention flocks were required to comply with a set of standard biosecurity measures. The biosecurity measures included a standard method of cleansing and disinfecting the poultry house prior to stocking, and a standard hygiene protocol followed by all personnel who entered the study houses during the target flock's life. The biosecurity measures specified included: dust removal by blowing; all internal surfaces washed with defined sanitizer; drying period between washing and disinfection > 6h; house dry before disinfection; all internal surfaces disinfected: specified product at defined dilution rate; broody chick equipment disposable or washed/disinfected in main house at the same time; adjoining rooms to poultry house hand washed and disinfected (if not in main wash/disinfection programme); water system cleaned; disinfected for 1 h; iodine-based disinfectant; concrete areas on the site disinfected before litter is placed; procedures during the study period; two boot dips: on entry to anteroom and on entry to main house; boot dip disinfectant as specified; use only dedicated boots and overall in study house; separate (chalk line or bench) clean area of anteroom next to house entrance; and hand sanitizer provided. The farm staff for all flocks filled out questionnaires, throughout the flock rearing times, specifying which biosecurity measures were undertaken. Staff for intervention flocks (n=13) were given instructions on biosecurity requirements and reminded on a regular basis to encourage compliance while the staff for control flocks (n=25) received no such instructions or reminders. All flocks were monitored weekly for *Campylobacter* colonization and at 42 days of age the risk of infection was reduced by over 50% in the intervention flocks. The results of this study appear to indicate that the rigorous application and policing of biosecurity measures can significantly reduce the prevalence of *Campylobacter*-positive flocks, at least in the UK. This information has, therefore, been used on the UK data as the basis of an intervention input in CAMO (Chapter 5).

4.2.1.2. Fly screens

A relatively new approach to increase a higher level of biosecurity is to prevent ingress of insects into the broiler house. Intervention studies undertaken in Denmark (Hald *et al.*, 2007) have indicated that the exclusion of flying insects from the house can significantly reduce the *Campylobacter* flock positivity during the seasonal peak. A study carried out from June to November 2006 comprised 20 intervention houses (on 11 farms) with 50 flocks and a matched group of 25 control houses (on 13 farms) with 70 flocks. The main matched criteria between the study group and control group were the prevalence of *Campylobacter*-positive flocks produced in the houses during the three previous years (2003-2005), which for the study group was 51.6% and for the control group 51.7%. All access points in the broiler houses for insects larger than a few millimetres, were secured by custom-built screens made of netting. The main focus was placed on the ventilation inlets. During the study period, the *Campylobacter* prevalence dropped in the netted houses compared with the control houses. At the flock age of 35-42 days immediately before catching, the prevalence was 45.5% in the control houses and only 7.7% in the netted houses. Sampling at slaughter showed that the prevalence in flocks remained unchanged in the control houses at 51.4% positive, whereas the prevalence dropped to 15.4% in the netted houses. Furthermore, there was a significant delay in onset of colonization by 2 weeks in the netted houses.

It seems important to fly-net all the houses on a farm to achieve effective protection against *Campylobacter*, as nearby non-netted houses can pose a higher risk compared to the netted houses; not by insect transmission, but by other transmission routes on the farm, probably mainly by personnel traffic. The above study, therefore, had intervention and control houses on separate farms, and on those study farms with two houses, both houses were netted. A pilot study from April to November 2004 included five Danish broiler farms each with two houses (one intervention and one control house on each farm). 18 flocks were reared in the netted houses and 18 in the non-netted houses (Hald *et al.*, 2005). In the non-netted houses eight flocks were *Campylobacter* positive before depopulation for slaughter while only five flocks in the netted houses were positive. The netted houses were infected later in the rearing period than the non-netted houses, as the netting caused a lag in introduction of *Campylobacter* of two weeks ($P<0.0001$). As strains isolated in *Campylobacter*-positive flocks of the netted houses were identical by pulse field gel electrophoresis (PFGE) to strains of flocks in the corresponding non-netted house, transmission from the non-netted houses was considered probable.

Fly netting has also recently been carried out in Iceland, but using a more practically oriented approach very different from the Danish studies. In this approach, 'problem' farms having an historically high *Campylobacter* prevalence were identified and all houses on these multi-house farms were fly netted in 2008 (Lowman *et al.*, 2009). On three farms with 19 broiler houses belonging to Company A, the prevalence dropped from 48.3% to 25.6% during the period June-September. On another farm with 16 houses belonging to Company B the prevalence dropped from 31.3% to 17.2% during the period July-September. Since 2008 the netting of broiler houses in Iceland has been continued and optimized with a concomittant further decrease in prevalence.

In conclusion, netting of broiler houses with the aim of excluding insects has shown significant reductions in summer prevalences in broiler flocks in Denmark and Iceland. However, further work is needed to demonstrate the effect of fly nets in countries with different climatic conditions. Nevertheless, the success in Denmark of using fly screens has been used on the Danish data as the basis of an intervention input in CAMO (Chapter 5).

4.2.2. Drinking water

Another factor linked to biosecurity is the quality of the drinking water. Several studies have found that drinking water of poor quality, (i.e., untreated water from wells) is related to an increased risk of a flock being positive for *Campylobacter* (Guerin *et al.*, 2007; Lyngstad *et al.*, 2008; Sparks, 2009; Stern and Pretanik, 2006). As *Campylobacter* is frequently isolated from untreated water sources, this is not surprising.

Clearly, if the use of water of less than drinking water quality is the only option, then measures to treat the water on farm (e.g. by chlorination, filtration or UV irradiation) should be implemented even though the impact of such interventions on *Campylobacter* infection remains unclear (Gibbens *et al.*, 2001; Mohyla *et al.*, 2007; Pearson *et al.*, 1993). Routine chlorination is widely considered fully effective against *Campylobacter*. Although, in most MSs intensively-reared flocks are supplied with potable water, levels of chlorination can differ even between regions within individual MSs and relatively low levels of chlorination of flock drinking water (2 to 5 parts per million) are apparently ineffective in decreasing the prevalence of colonization by *Campylobacter* (Stern *et al.*, 2002). One explanation of the inconsistent results is that *Campylobacter* can persist within protozoa and biofilms where partial protection from disinfectants may occur (Cox and Pavic, 2010). Such environmental conditions are likely to occur regularly in poultry farm water reservoirs and lines. Thus, effective interventions to deliver *Campylobacter*-free water may require extremely rigorous and regular water line cleansing.

Because the effectiveness of drinking water decontamination as an individual biosecurity measure is unclear, this intervention was not investigated in CAMO.

4.2.3. Reduction of slaughter age

As indicated previously the prevalence of flock positivity is directly related to slaughter age. Thus slaughtering at a younger age should be an effective intervention. In countries like Sweden (Berndtson *et al.*, 1996), where the majority of flocks are harvested at 33-35 days of age, increasing the age of slaughter to 42-44 days increased the flock positivity by about two-fold and to 48-61 days by about four-fold. However, the effect of thinning on this linear increase in positvity is unstated. Nevertheless, the earlier slaughter accounts in large part for the lower positive flock prevalence in Scandinavia (Berndtson *et al.*, 1996; Kapperud *et al.*, 1993).

Data from the EU baseline survey (EFSA, 2010b) showed that in a multivariate analysis, the risk of colonization by *Campylobacter* increases approximately two-fold (OR=1.98, 95% CI [1.66;2.35]) for every 10 days that the birds are older. The multivariate model accounted for concurrent effects of thinning and season. Even though all data were included in the analysis, the results can only be considered valid for indoor production and slaughter ages up to 50 days, as this was the dominant production system in all MSs.

Because this effect was so apparent in previous observations and well recognised by expert opinion, the EU baseline surveydata was used as an input into CAMO (Chapter 5) to investigate the effect of reducing slaughter age on public health. However, because the EU baseline survey data derives from many flocks managed under varying conditions the output may not equate with less complex studies undertaken in one country.

4.2.4. Discontinued thinning

As previously indicated (chapter 4.1.3.), thinning can constitute a high risk of flock infection.

Stopping thinning would reduce the risk of *Campylobacter* introduction into a house, both due to the lowering of the slaughter age of one or more slaughter batches (assuming that all birds will be slaughtered at the same time as the first thinning used to happen), and due to the reduced traffic in the house during the life span of the flock.

The data from the EU baseline survey (EFSA, 2010b) show that in a multivariate analysis, slaughter batches of previously thinned flocks are at a significantly higher risk of colonization by *Campylobacter* (OR=1.74, 95% CI [1.36;2.24]) than batches that had not been subject to previous thinning. The multivariate model accounted for concurrent effects of slaughter age and season.

Because discontinued thinning would be a relatively simple intervention to implement, it was therefore incorporated into CAMO (Chapter 5).

4.2.5. Bacteriocins

Bacteriocins are proteinaceous toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strain(s). Most bacteriocins exhibit antibacterial activity only against bacteria closely related to the producer strain but a few display broad-spectrum activity. Lin (2009) has reviewed anti-*Campylobacter* bacteriocins for potential use in reducing the numbers of *Campylobacter* (*jejuni* as well as *coli*) in poultry. Currently four purified bacteriocins reduce *Campylobacter* colonization in poultry: SRCAM 602 from *Paenibacillus polymyxa* NRRL B-30509 (Stern *et al.*, 2005), OR-7 from *Lactobacillus salivarius* (Stern *et al.*, 2006), and E-760 and E 50–52 from *Enterococcus* spp. (Line *et al.*, 2008; Svetoch *et al.*, 2008).

In the study by Stern *et al.* (2005), one-day-old chicks were orally inoculated with *C. jejuni* and from 7 to 10 days of age, the chickens were provided with either standard broiler starter feed (control) or this feed modified with purified SRCAM 602. All 10-day-old control chickens (n=10) were colonized in the caecum with 6.6–8.3 $\log_{10}$ CFU of *Campylobacter* per g, while all treated chickens contained undetectable numbers (< 2 $\log_{10}$ CFU/g). Using the same experimental set-up for OR-7, mean reductions in the *Campylobacter* counts from 10-day-old chickens from 6.6-8.3 $\log_{10}$ CFU/g caecal contents to undetectable levels were achieved (Stern *et al.*, 2006). Enterococcin E-760 also reduced the *Campylobacter* colonization of naturally-infected broilers of market-age when administered for four days prior to analysis: *Campylobacter* counts in the control group were 5.15-8.36 $\log_{10}$ CFU/g while after treatment undetectable levels were again obtained (Line *et al.*, 2008). Svetoch *et al.* (2008) administered bacteriocin E 50–52 to young chicks using the same approach as Stern *et al.* (2005), but the chicks were killed on day 15 instead of day 10. Again high levels of *C. jejuni* were found in the control chicks (8.40 $\log_{10}$ CFU/g of caecal contents), while no *Campylobacter* was detected in the treated group. As this result was obtained 8 days after termination of the bacteriocin treatment, the authors (Svetoch *et al.*, 2008) suggested that the bacteriocin treatment completely eliminated *C. jejuni* from the chicken intestine. E 50–52 was also very effective in reducing *Campylobacter* colonization of naturally infected broilers at market-age when administered via the drinking water. A 5.1 to 5.85 $\log_{10}$ unit reduction of *C. jejuni* colonization was achieved after one day of treatment (Svetoch *et al.*, 2008).

Thus, it seems that bacteriocins, adminstered just before slaughter, can reduce *Campylobacter* colonization in the chicken caecum to undetectable levels. However, field validation of the *in-vivo* bacteriocin trials has not yet been conducted and current estimates are based on limited experimental studies. Because of this lack of validation this potential intervention was not modelled even though the data appear promising. Large-scale field trials are now needed to determine the practicality of bacteriocin treatment in a commercial poultry environment. Also, there are logistical issues regarding the scale-up of bacteriocin production and purification, which to date appear complex and would require timely application in an industry which tends to need flexible production approaches.

4.2.6. Bacteriophages

The lytic activity of bacteriophages can be used to kill *Campylobacter*. Such phages usually have a narrow host spectrum, and they do not interact with other bacterial species (e.g. other members of the gut flora). Phages attach to and enter bacterial cells through a receptor (e.g. protein, LOS). Phage multiplication then occurs and the progeny phages are released by lysis of the host bacterium to infect another host. If the receptor changes, *Campylobacter* can become resistant to the phage. However, in contrast to antimicrobials, phages are non-static organisms and evolve rapidly alongside their bacterial hosts, which may explain why resistant *Campylobacter* bacteria remained a small population in phage-treated chickens in an experimental setting (El-Shibiny *et al.*, 2009).

For the control of *Campylobacter*, bacteriophages are usually proposed as therapeutic for, rather than preventative of, flock colonization. The strategy of such intervention is to treat flocks two to three days prior to slaughter. Assuming that treated flocks will not be fully cleared of *Campylobacter*, the prevalence of positive flocks will be unchanged after treatment but there will be a reduction of the *Campylobacter* numbers in caecal contents and faeces. Reductions in caecal *Campylobacter* levels of 0.5-5 $\log_{10}$ have been described (El-Shibiny *et al.*, 2009; Loc Carrillo *et al.*, 2005; Wagenaar *et al.*,

2005) and are dependent on the timing, dose of phages and the phage-*Campylobacter* combinations. In addition, the efficiacy of phage therapy depends on the presence of threshold levels of susceptible *Campylobacter*, so effectiveness might be reduced if treatment was applied in the early stages of colonization.

The phage-host co-evolution is complex. Experiments have indicated that the ecological fitness of *Campylobacter* strains is related to their susceptibility to phages. Whether the large scale and global use of phage therapy would result in a change in the epidemiology of targeted *Campylobacter* strains (Scott *et al.*, 2007) is, as yet, difficult to assess.

Currently there is no specific EU-regulation regarding the use of phages in primary production. Two options are available to consider them either as a feed-additive or a veterinary drug.

Under experimental conditions the use of phages appears promising in reducing *Campylobacter* numbers; however, a number of practical aspects require consideration. The diversity of *Campylobacter* receptors means that multiple phage populations will be required, which increases the production complexity. The safety and practical aspects of the bulk culture of matching multiple, potentially pathogenic, *Campylobacter* strains for phage production is a serious issue. Theoretically, the application should be shortly before slaughter for maximum effect in the food chain, which generates logistical problems. Also the development of resistant strains, and their distribution into the farm environment could be complicating factors while continuous monitoring of phage profiles and susceptibility of those *Campylobacter* strains colonizing the flocks would be necessary. Because of these issues, the use of bacteriophages as currently envisioned has limited practicality. Until these limitations are addressed and field trials can be undertaken this intervention was considered to be still in the early stages of development and therefore not suitable for modelling. A generic modelling approach to evaluate the public health risk reduction due to reducing the number of *Campylobacter* in bird's intestines was performed to evaluate the potential benefit of interventions with this general aim.

4.2.7. Vaccination

Assuming that biosecurity can never be fully effective, the vaccination of poultry to reduce susceptibility to *Campylobacter* could be used to support biosecurity measures. Vaccination might reduce or even prevent colonization and in both cases would affect the numbers of organisms entering the food chain and the environment.

Serum and mucosal antibodies directed against *Campylobacter* are generated by chickens during colonization. These antibodies are not therapeutic during the short life of most intensively-reared broilers (about 6-7 weeks) but are, at least in part, protective aganst subsequent experimental challenge (Cawthraw *et al.*, 1998). Maternally-derived antibodies also provide protection against *Campylobacter* colonization (Cawthraw and Newell, 2010; Sahin *et al.*, 2003) and this protection is apparently sustained for up to two weeks. Actively acquired immunity may also be therapeutic as reduced *Campylobacter* numbers in the caecum and a decreased proportion of colonized birds has been reported to occur from eight weeks post-colonization (Newell and Fearnley, 2003). However, to date, the generation of protective immunity by vaccination has proven problematic.

Proof of principle that *Campylobacter* antibodies induced by vaccination in chickens can have protective properties was first shown by Stern *et al.* (1990). Subsequently many vaccination regimens and strategies have been tested (de Zoete *et al.*, 2007), but most studies have been poorly reproducible. The problems with vaccination include mass delivery of an effective vaccine to immunologically immature birds in order to induce a sufficiently rapid response at the gut mucosal surface to protect the bird from challenge within 2-3 weeks of hatching. Crucial in the vaccine development is the protective antigen used. Most research to date has focused on flagellin as the protective antigen. Experimental studies have been performed with whole-cell vaccines, flagellin subunit vaccines and a limited number of other antigens (de Zoete *et al.*, 2007).

So far the protection induced by vaccination with sub-unit or killed vaccines, has not substantially reduced colonization in chickens. Recent studies with live vaccines using *Salmonella* vectors are more promising, though reproducibility, safety and licensing may be issues. Current vaccine research is investigating alternative protective antigens and novel approaches, such as mucosal adjuvants or cytokines, to boost the chicken immune system.

Once an effective vaccination system has been developed then the efficacy of any vaccine candidate can be tested under experimental conditions. Dose-response experiments will show the protection against a challenge and what reduction in *Campylobacter* numbers can be observed in animals that become colonized. However, the efficacy of such a vaccine under field conditions, in terms of preventing flock colonization or reducing levels of colonization in individual birds, is hard to predict because the naturally-occuring exposure dose to broilers, in terms of both of numbers of organisms and their physiological status, remains unknown. Thus, at present, estimates of either the preventive or the therapeutic effects cannot be made and therefore this potential intervention has not been modelled.

4.2.8. Feed and water additives

Feed additives can comprise chemical additives like organic acids as well as biological agents as used in the competitive exclusion (CE) concept. The latter one uses defined cultures or undefined microorganisms from the guts of healthy animals (in this case poultry) to prevent *Campylobacter* subsequently occupying its specific niche. To date no defined cultures which exclude *Campylobacter* have been identified. However, undefined gut flora appears more successful. Such flora have been experimentally administered to birds immediately after hatching and the birds subsequently challenged with *Campylobacter*. Stern *et al.* (2001a) described a significant reduction of *Campylobacter* in the faeces with two different CE approaches, compared to a control group without CE. This is in agreement with earlier investigations by Mead *et al.* (1996). However, because of the lack of standardisation of such undefined CE preparations, modelling to evaluate the efficacy of such and intervention was not considered possible.

Although short chain fatty acids do not appear to be effective in reducing *Campylobacter* shedding in broilers, butyrate was considered effective (Van Deun *et al.*, 2008). In addition, caprylic acid in concentrations of 0.7% can give a reduction of up to 3-4 logs in the numbers of *Campylobacter* in broiler faeces (de los Santos *et al.*, 2008a; 2008b; 2009; 2010).

In contrast, the addition of any of aproic, caprylic, and capric acid to the feed of broilers, starting 3 days before slaughter, failed to reduce *Campylobacter* colonization levels in 27-day-old broilers experimentally infected with *C. jejuni* at 15 days of age. This was consistent with the failure of sodium caprate to reduce *Campylobacter* counts using a caecal loop model. When time-kill curves were conducted in the presence of chick intestinal mucus, capric acid was less active against C. *jejuni*. At four millimoles (mM), all bacteria were killed within 24 h. Thus, despite the marked bactericidal effect of medium-chain fatty acids in vitro, supplementing these acids to the feed did not reduce ceacal *Campylobacter* colonization in broiler chickens under the applied test conditions, probably due to the protective effect of the mucus layer in this study (Hermans *et al.*, 2010).

In another study by Skanseng *et al.* (2010) chickens were offered feed supplemented with different concentrations and combinations of formic acid and/or potassium sorbate. There was little or no effect on *C. jejuni* colonization levels in chickens that were given feed supplemented with formic acid alone. A combination of 1.5% formic acid and 0.1% sorbate reduced the colonization of *C. jejuni* significantly, while a concentration of 2.0% formic acid in combination with 0.1% sorbate prevented *C. jejuni* colonization in chickens.

Hilmarsson *et al.* (2006) found that a combined treatment with monocaprin in water and feed did not prevent spread of *Campylobacter* from artificially infected to non-infected 24-day-old chickens, but *Campylobacter* counts in cloacal swabs were significantly reduced, particularly during the first two days of treatment.

Application of organic acids via drinking water has produced various results. Chaveerach *et al.* (2004) found that whilst the acid-treated drinking water remained free of *Campylobacter* throughout the study the consumption of acidified drinking water had a limited effect on the microflora in the chicken intestinal tract compared to the control group. *Campylobacter* spp. were reduced by an average 0.54 to 2 $\log_{10}$ cycles in the faeces, but with a high standard deviation. The drinking water of the acid-free control group was found contaminated with *Campylobacter*. Survival under acid conditions may lead to viable but non-culturable (VNBC) cells (Chaveerach *et al.*, 2003; Sparks, 2009). In another study the prevalence of *Campylobacter* spp. in broiler crops was reduced from 85% to 62% by application of lactic acid in drinking water during the ten hours of feed withdrawal (Byrd *et al.*, 2001). Animal health and welfare were not affected by the acid application. A pH of around 4.0 proved to be most effective against *Campylobacter* when formic, acetic, propionic, and hydrochloric acids were tested in vitro as additives to a mixture of water and feed, alone or in combination (Chaveerach *et al.*, 2002).

Monocaprin (1-monoglyceride of capric acid) has been shown to be effective as a diluted emulsion in water, with or without Tween surfactants (Thormar *et al.*, 2006). They may, therefore, be used to eliminate or reduce numbers of viable *Campylobacter* in contaminated drinking water for chickens. Furthermore, there seemed to be a synergistic effect of monocaprin and organic acids in killing *Campylobacter* (Thormar *et al.*, 2006). According to this study, addition of monocaprin emulsions to the drinking water and feed of broiler chickens had no adverse effect on their health or growth rate.

Chlorination of drinking water was found to be a protective factor in premises where water quality was poor, e.g. from non-approved sources. Furthermore, treating drinking water over a period of time (usually several days) led to a reduction of the overall microflora including *Campylobacter* spp. by up to 2 $\log_{10}$ cycles in faecal counts. Substances used were organic acids (e.g. caprylic acid) and fatty acids (monocaprin).

These interventions were not included in the CAMO because the results of adding organic acids to feed or water have given inconsistent results in terms of numbers and prevalence of *Campylobacter*.

4.2.9. Selective breeding

The effect of host lineage on the susceptibility of chickens to *Campylobacter* has been investigated using inbred chicken lines. This effect was first recognised in a chick embryo infection model (King *et al.*, 1993) and later confirmed in an oral challenge model (Boyd *et al.*, 2005). Genetically endowed resistance can apparently reduce experimental colonization levels by 10-100-fold. With the recent availability of the chicken genome sequence, the genes involved in such susceptibility are now being identified. These observations have led to the suggestion that selective breeding could be used to breed *Campylobacter*-resistant chickens (Kaiser *et al.*, 2009). However, as a potential intervention, selective breeding programmes, especially in poultry previously selected for high meat or egg production, must be considered as a very long term goal. Therefore, this potential intervention was not modelled.

4.3. Risk factors for *Campylobacter* at transportation and before slaughter

As birds are prepared, caught, loaded, transported to processing plants and finally slaughtered, they undergo stresses that may affect not only broiler meat quality, but also its microbiological status. Feed, and sometimes water, is usually withdrawn several hours before the birds are caught and loaded into crates for transportation to the slaughter-house; no water is available in the crates. Bird contamination with *Campylobacter* may increase significantly during catching and placing the birds in crates for transportation to the slaughter-house (Slader *et al.*, 2002). The plastic crates commonly used to transport live birds from the farm to the processing plant are known to be a source of contamination and cross-contamination (Allen *et al.*, 2008a; 2008b; 2008c; Slader *et al.*, 2002).

During transportation to the slaughter-house, and while waiting to be slaughtered, the stress of crowding birds in close proximity in crates is likely to further disseminate *Campylobacter* contamination present in faeces, and on the skin and feathers or released from the gastrointestinal tract during this process (Stern *et al.*, 2001b; Whyte *et al.*, 2001a). Contaminated birds spread

contamination to the environment and cross-contaminate other birds. Strains of *Campylobacter* present in faeces of one lot of birds may be recovered from carcasses of subsequent lots (Arsenault *et al.*, 2007).

While time spent in the waiting area before slaughter should be kept to a minimum, sometimes it may extend to many hours or even overnight (Bayliss and Hinton, 1990). Unlike lairage provided for red-meat animals, 'lairage' for poultry is not beneficial, because while waiting to be slaughtered the birds continue to be confined in crowded crates, deprived of water as well as food, and spreading of contamination among them continues. In addition, holding time before slaughter should be minimized for welfare reasons. Care should be taken to protect the birds from direct sunlight and to avoid overheating or cold and wet conditions in the waiting area. Arsenault *et al.* (2007) found weather during transportation to slaughter was associated with carcass contamination with *Campylobacter* and that contamination was higher in lots of birds slaughtered at the end (i.e., Wednesday or Thursday; as no slaughter was performed on Friday) than earlier in the week.

Campylobacter strains predominating on live birds as they arrive at the processing plant are not necessarily the same as those isolated from processed carcasses. This may indicate cross-contamination occurring during processing or differences in strain tolerance to the processing conditions and the factory environment (Slader *et al.*, 2002). Newell *et al.* (2001) isolated from processed, previously negative broilers the same subtype of *Campylobacter* that was found on transport crates, used to transport the birds to the factory. This contamination could have been introduced during transportation or at the processing factory. Berrang *et al.* (2003) found that *Campylobacter* negative birds acquired the pathogen on their feathers from transport cages. Results by Takahashi *et al.* (2006) indicated that chicken wing meat contamination resulted mainly from farm strain carryover.

In general it can be concluded that:

- External cross-contamination of birds with *Campylobacter* may occur during catching and placing the birds in crates, transportation to the slaughter-house, and holding before slaughter; undesirable effects increase with transportation and holding time.
- It is difficult to quantify the contribution of cross-contamination during transportation and holding time before slaughter to the eventual broiler carcass contamination because of the numerous variables and confounding factors involved.
- Therefore, it is important and useful to minimize transportation and holding time before slaughter for microbiological, product quality, economical, and animal welfare reasons.

4.4. Interventions against *Campylobacter* at transportation and before slaughter

4.4.1. Feed and water withdrawal

The length of time spent in transportation and waiting to be slaughtered varies considerably in Europe, and depends on the distance between the farm and the slaughter-house, and also on the organizational skills of the firm; ideally, slaughter-houses should be located near rearing farms. Feed, and sometimes water (at least during transportation), withdrawal is practiced before shipment to the slaughter-house in order to reduce the contents of the gastrointestinal tract, and consequently the volume of faeces excreted during transportation. The expectation is that this contributes to lower *Campylobacter* numbers in the environment and on carcasses (Bilgili, 2002; FAO/WHO, 2009a, 2009c; FSIS/USDA, 2008a; Keener *et al.*, 2004; Thompson and Applegate, 2008). However, certain studies have reported increased potential for bird and carcass contamination with increased time of feed withdrawal (Bilgili and Hess, 1997; Keener *et al.*, 2004; Northcutt *et al.*, 2003). Jacobs-Reitsma *et al.* {, 1996 #11767} observed increases of 0.50 $\log_{10}$ CFU in *Campylobacter* counts in caecal contents of broilers subjected to feed withdrawal and transportation. However, the authors did not detect any significant difference in shedding of the organism as a result of feed withdrawal or four hours of containment in

transportation crates. Accordingly, it was suggested that feed withdrawal and holding time might be insufficient to counteract the effects of catching and transportation stress on *Campylobacter* excretion. Willis *et al.* (1996) found that levels of *Campylobacter* found in various sampling sites did not differ between broilers given water and those without, but were different at varied feed withdrawal times. It was also found that broilers not given water had overall *Campylobacter* prevalence 17% higher (70% compared to 53%) for cloacal samples but 5% lower prevalence in ceacal contents than broilers given water. Thus, it was concluded that carcass contamination could occur regardless of presence of water during feed withdrawal.

Warriss *et al.* (2004) observed that the gastrointestinal (except for the caeca) contents became more liquid during feed and water withdrawal; this might increase the likelihood of faecal contamination during evisceration. Zuidhof *et al.* (2004) reported that a 12-hour feed withdrawal period, including eight hours in the broiler house, was adequate for clearing the contents of the digestive tract and preventing carcass shrink. There are indications that the norepinephrine hormone secreted by stressed birds might stimulate multiplication of *Campylobacter* in the gut during transportation, thus leading to increased numbers on the carcass after processing of *Campylobacter* positive flocks (Cogan *et al.*, 2007).

The situation becomes more complicated as Stern *et al.* (1995) did not find significantly higher *Campylobacter* numbers in the caecal contents after simulated transportation lasting overnight, but found significantly higher numbers on the outside of the birds after actual or simulated transportation, presumably due to faecal contamination. These observations support the need to minimize the time birds spend in transit and waiting for slaughter. In studies by Northcutt *et al.* (2003) feed withdrawal affected *Campylobacter* presence on carcasses of only older broilers (56 days of age). In addition, it was reported that feed withdrawal may have increased counts of *Campylobacter* on pre-chilled carcasses but had no effect on counts of carcasses chilled with sodium hypochlorite solution. Specifically, Northcutt *et al.* (2003) found that carcass log counts (and positive samples) of *Campylobacter* were 2.1 (65/72) and 2.5 (66/72) after 0 and 12 hours of feed withdrawal, which included the one hour transportation and seven hour holding time in coops, respectively. Although these differences were statistically significant, a 0.4-$\log_{10}$ unit difference would most likely be practically insignificant.

Warriss *et al.* (2004) reported that the frequency of defaecation was reduced after about four hours of feed or feed and water withdrawal, and that the quantity of gut contents was diminished in the proximal parts of the gut (crop, gizzard and small intestine) to optimal levels after about 12 hours. Withdrawing water as well as feed delayed emptying of the crop. According to Northcutt *et al.* (1997) the integrity of the intestines decreased after 12 to 14 hours of feed withdrawal, as indicated by heavy intestinal sloughing. The authors concluded that long feed withdrawal times could cause a rapid deterioration in the condition of the viscera, which would increase the likelihood of carcass contamination. Others (Corrier *et al.*, 1999; Ramirez *et al.*, 1997) have concluded that feed withdrawal may lead to increases in *Salmonella* in the broiler crop due to consumption of litter by the broilers. Feed withdrawal also caused a significant increase in *Campylobacter*-positive crop samples in seven of nine houses tested, but it did not significantly affect the frequency of *Campylobacter* isolation from the caeca (Byrd *et al.*, 1998); this may result in increased *Campylobacter* contamination of carcasses at processing. A related concern is that the reduced intestinal integrity associated with feed withdrawal may increase susceptibility to infection (FSIS/USDA, 2008a, 2008b; Nijdam *et al.*, 2005; 2006; Thompson and Applegate, 2006). Wesley *et al.* (2009) reported overall increases in *Campylobacter* isolated from the gallbladder and the crop of turkeys at the abattoir compared with on-farm levels, and suggested that this could be associated with a decline in lactic acid in the emptied crop. Thus, additional adjustments and modifications in bird management practices may be necessary in order to optimize the benefits of feed withdrawal and to avoid potential negative effects.

Findings have indicated that, with emptying of the crop during extended feed withdrawal, there is a decrease in the number of lactic acid producing bacteria in the crop and an associated increase in crop pH which may be related to a reduction of activity against enteric pathogens in the crop (Hinton *et al.*,

2000a; Mead, 2000). This is supported by findings that supplementation of drinking water with organic acids during feed withdrawal was associated with decreased post-harvest broiler crop and carcass contamination with *Salmonella* and *Campylobacter* (Byrd *et al.*, 2001). A related approach is to feed broilers with carbohydrate based "cocktails" (liquid feed instead of water during feed withdrawal before transport) or special replacement diets (e.g. whole grain) before initiation of feed withdrawal in order to limit pathogen growth in the crop by stimulating the growth of natural microflora (Delezie *et al.*, 2006; Farhat *et al.*, 2002; FSIS/USDA, 2008a, 2008b; Hinton *et al.*, 2000b; Nijdam *et al.*, 2006; Northcutt *et al.*, 2003; Rathgeber *et al.*, 2007). Hinton *et al.* (2002) found that broilers fed sucrose cocktails had significantly lower levels of *Campylobacter* and *S*. Typhimurium in their crops than those given equal concentrations of glucose. Northcutt *et al.* (Northcutt *et al.*, 2003) found *Campylobacter* counts of 2.0-3.9 and 2.5-3.9 $\log_{10}$ CFU/ml carcass rinse of broilers exposed to 0-12 hours of feed withdrawal and provided nothing or replacement diets, respectively. Thus, it was concluded that type of feed or length of feed withdrawal did not affect *Campylobacter* counts on carcasses. Feeding *Saccharomyces boulardii*, a non-pathogenic yeast, to broilers reduced *Salmonella* but not *Campylobacter* colonization in the caeca (Line *et al.*, 1998). In general, providing birds with drinking water additives such as lactic acid during feed withdrawal or enhancing acid production in the crop may lower post-harvest crop contamination levels but does not seem to affect levels in the caeca (FSIS/USDA, 2008a, 2008b). Slaughtering birds eight to 12 hours after feed withdrawal has been recommended as the optimum practice for reducing the likelihood of carcass contamination with faecal material or ingesta (Bilgili and Hess, 1997; Delezie *et al.*, 2006; FAO/WHO, 2009a, 2009c; FSIS/USDA, 2008a, 2008b; Rathgeber *et al.*, 2007; Thompson and Applegate, 2008; Zuidhof *et al.*, 2004). However, early feed withdrawal makes the internal organs more fragile and allows the crop and cloaca to tear easily during processing (FSIS/USDA, 2008a, 2008b). Twelve hours of feed withdrawal resulted in an optimal combination of gastrointestinal clearance and carcass yield (Zuidhof *et al.*, 2004).

The following conclusions can be drawn for feed withdrawal:

- Feed withdrawal programmes vary among countries due to differences in production practices and volumes, slaughter procedures, and inspection programs.

- Considerations important in the selection and implementation of feed withdrawal programmes include extent of visible and microbiological carcass contamination expected, associated live bird weight losses and yield, and time needed for transportation of birds from the farm to the slaughter-house, as well as anticipated time spent in the waiting area before slaughter. Feed withdrawal times should be such that, in addition to physical emptying, the integrity of the gastrointestinal tract is also maintained.

- Feed withdrawal times longer than 12 hours could cause deterioration in the condition and integrity of the viscera and increase the fluidity of gastrointestinal contents, which could increase the potential for faecal contamination of carcasses.

- Modifications in broiler management practices related to diet might help in optimizing the benefits of feed withdrawal and to avoid potential negative effects. Providing birds with drinking water additives such as lactic acid during feed withdrawal or enhancing acid production in the crop may lower post-harvest crop contamination level.

- Feed withdrawal, transport and holding time before slaughter should be between eight and 12 hours; the 12 hour limit should not be exceeded because it is the maximum feed withdrawal time allowed by EU regulation for animal welfare reasons.

- Overall, available data are inadequate and the complexity of variables and confounding factors involved make it difficult to assess the effect of interventions such as feed withdrawal or good hygiene practices during transportation and holding before slaughter on the final microbiological contamination of carcasses.

4.4.2. Cleaning and disinfection of crates

In Europe, birds are usually transported from the farm to the processing plant in modular systems, which typically consist of metal frames into which are slotted plastic drawers (crates). The crates are made of high-density polypropylene with extensive webbing to produce a fine mesh construction for ventilation. Transport crates can be contaminated with faeces of *Campylobacter*-infected flocks, while broilers from uninfected flocks, and subsequently carcasses, can become externally contaminated with *Campylobacter* due to exposure to faeces during transportation and holding in the transportation crates (Berrang *et al.*, 2003; Bull *et al.*, 2006; Hansson *et al.*, 2005; Hiett *et al.*, 2002; Rasschaert *et al.*, 2007; Slader *et al.*, 2002). There is limited evidence that birds remain in contaminated transport crates long enough for intestinal colonization to occur (Rasschaert *et al.*, 2007).

Both modules and crates should be washed and disinfected after each use in order to reduce cross-contamination between *Campylobacter* positive and negative flocks and decrease the amount of contamination introduced in the slaughter facility (Allen *et al.*, 2008a). This is usually done using mechanised cleaning systems. Washing transport cages with water and disinfectant can certainly reduce the level of *Campylobacter*, but it is not very reliable and does not completely eliminate the pathogen. A number of studies have shown that many crates are still contaminated with *Campylobacter* and/or *Salmonella* after cleaning because they are very difficult to clean and sanitize (Corry *et al.*, 2002; Hansson *et al.*, 2005; Stern *et al.*, 2001b).

In practice, washing procedures can break down due to human error, incorrect application of chemicals, or transport crates become re-contaminated with *Campylobacter* even after they are washed and disinfected at the factory {Humphrey, 2002 #11768}. Thus, crates constitute a potential source of contamination and cross-contamination of the exterior of previously *Campylobacter*-negative birds transported for slaughter, as well as of the factory environment and processed carcasses (Allen *et al.*, 2008a; Bailey *et al.*, 2001; Corry *et al.*, 2002; Newell *et al.*, 2001; Rigby *et al.*, 1980; Slader *et al.*, 2002). In general, *Campylobacter* have been found in chicken transportation crates prior to use, even after washing and sanitizing (Stern *et al.*, 2001b)) in high numbers (up to 7 $\log_{10}$ CFU), of multiple genotypes, and with little association to any visual assessment of cleanliness (Allen *et al.*, 2007b). Berrang *et al.* (2004a) found no decrease in *Campylobacter* numbers on transport cages during eight hours of storage, but they also concluded that storing cages for 48 hours between uses resulted in lowered numbers in faeces. However, it was speculated that due to cage cost and space requirements, routine cage storage between uses would not be practical.

Slader *et al.* (2002) found that samples of unwashed, washed, and washed and disinfected crates were *Campylobacter* positive at the rates of 6/6, 5/5 and 2-5/5, respectively. Most probable number (MPN) *Campylobacter* counts detected on washed crates and washing water were <40-3,600 and <0.2 - >180, respectively. Thus, it was demonstrated that the cleaning process had little, if any, effect on the microbiological status of transport crates. Since organic matter may not be completely removed from crates, especially when cleaning is inadequate, it protects contamination embedded in it during washing and disinfection of crates. Hansson *et al.* (2005) reported that *Campylobacter* were isolated from 57% of 122 batches of washed and disinfected transport crates, some of which then contaminated farm-negative flocks during transport to the abattoir. Complete removal of faeces from transport crates is difficult, time consuming, costly, and requires large amounts of water. Residual faeces make washing systems ineffective. In addition, use of contaminated recycled water in washing and incorrect sanitizer levels lead to further failures in crate washing effectiveness (Corry *et al.*, 2002).

In general, presently used procedures for cleaning plastic crates or drawers used to transport live birds to the abattoirs are proven inadequate for eliminating microbial contamination. Correct use of disinfectants at recommended concentrations, after crate washing, reduces *Campylobacter* contamination prevalence and concentration; however, even though *Campylobacter* is generally considered sensitive to disinfectants, treatments do not completely eliminate the pathogen from crates (Avrain *et al.*, 2003; Blaser *et al.*, 1986; Newell *et al.*, 2001; Peyrat *et al.*, 2008; Slader *et al.*, 2002; Trachoo and Frank, 2002; Wang *et al.*, 1983). A reason for the inadequate cleaning and disinfection of transport crates is their design (Allen *et al.*, 2008a). Crates have many niches that can trap organic

material and microbes. In addition, long-term use damages crate surfaces and creates more harborage sites for accumulation of dirt and formation of biofilm {Burton, 2002 #11769}. Furthermore, the cleaning process is usually performed in a limited and crowded space, which often leads to short cleaning cycles (Allen *et al.*, 2008a). Allen *et al.* (2008a) examined the microbiological contamination of crates before and after factory cleaning at three different processing plants and found that *Campylobacter* counts before and after cleaning were 5.6-6.9 and 2.9-5.7 CFU/crate base, respectively. Commercial crate cleaning systems are inadequate because they recycle most of the wash-water, but since disinfection is not complete, even adding fresh water, the impact would be limited {Burton, 2002 #11769}.

For better control of contamination, it is important to design crates that are easy to clean and made of non-corrosive material. In addition, it would be beneficial for cleaning and disinfection to be performed in appropriate facilities separated from the processing factory (Allen *et al.*, 2008a; 2008c). Another modification in crate washing systems could be inclusion of brushes in the crate-soaking tank in order to enhance removal of faeces through mechanical action. More frequent replacement of crate washing water would reduce the level of organic material present, but additional detergent would be needed (Slader *et al.*, 2002). In general, it should be possible to improve crate cleaning by modifying existing cleaning approaches. Examples of other modifications could include use of biodegradable crates or use of disposable crate liners for avoidance of cross-contamination among loads of birds (Slader *et al.*, 2002), and drying of cleaned crates before reusing them to eliminate *Campylobacter* (Berrang and Northcutt, 2005). All these approaches, however, are likely to be costly {Burton, 2002 #11769}. Recently, a patented (International Application PCT/US2009/044815) formulation of levulinic acid and sodium dodecyl sulfate (SDS) has been presented as highly effective in reducing *Salmonella* on transport crates (Anonymous, 2009). Application of ultrasound during crate cleaning, not only improved cleaning by loosening attached soil, but it may also have a synergistic effect with heat in killing microbes. This approach needs further evaluation and development (Allen *et al.*, 2008a). Such approaches might decrease the need for better cleaning and disinfecting approaches but they could increase costs (Slader *et al.*, 2002).

Relative to cleaning and disinfection of broiler transportation crates, it is concluded:

- *Campylobacter* have been routinely detected in chicken transportation crates prior to use, even after washing and sanitizing, in high numbers (up to 7 $\log_{10}$ CFU per crate), of multiple genotypes, and with little correlation to any visual assessment of cleanliness.

- Washing cages or crates with water and disinfectant can certainly reduce the levels of *Campylobacter*, but it is not very reliable and does not completely eliminate the pathogen. Crates, as presently designed, have many niches for accumulation of contamination, exacerbated by wear after repeated use, which damages crate surfaces and creates more sites for accumulation of dirt and biofilm formation. They are therefore very difficult to clean and sanitize.

- Thus, crates constitute a potential source of contamination and cross-contamination of the exterior of previously *Campylobacter*-negative birds transported for slaughter, as well as of the factory environment and processed carcasses.

- Improvement of crate washing and disinfection procedures should reduce cross-contamination between *Campylobacter* positive and negative flocks and will decrease the amount of contamination introduced in the slaughter facility.

- Overall, available data are inadequate and the complexity of variables and confounding factors make it difficult to quantify the effect of transportation crate washing on the final microbiological contamination of carcasses.

- The contribution of soil from transportation crates on external bird contamination should be further evaluated and effective interventions should be sought in order to minimize contamination increases and cross-contamination among birds and flocks.

4.5. Risk factors for *Campylobacter* during slaughter, dressing and processing

4.5.1. *Campylobacter*-colonized batches

In the EU-level multivariable regression analysis of the results of the EU-wide baseline survey of *Campylobacter* in broiler carcasses executed in 2008, a positive association between the prevalence of *Campylobacter*-colonized batches and the frequency of *Campylobacter*-contamination of the broiler carcasses was observed (EFSA, 2010b). A *Campylobacter*-colonized batch was about 30 times (adjusted OR: 28.62; 95% CI [20.39;40.17]) more likely to yield a *Campylobacter*-contaminated carcass. This positive association is not surprising, because contamination of the carcass with *Campylobacter* from the intestines during slaughtering process cannot be avoided completely. Since broiler carcasses are a food product on which the skin of the animal remains, carry-over of pre-existing faecal contamination of the skin through the slaughter process is also likely. In addition, analysis of the EU results showed that batches of broilers whose intestines were colonized with *Campylobacter* yielded carcasses with high numbers of *Campylobacter*.

4.5.2. Slaughter-house / slaughtering practices and hygiene

In the same survey (EFSA, 2010b) was observed that the risk for contamination of carcasses with *Campylobacter* and for high *Campylobacter* counts on carcasses varied significantly between countries and among slaughter-houses within countries even when other associated factors such as the prevalence of *Campylobacter*-colonized batches, were accounted for. These findings indicate that some slaughter-houses are more capable than others in preventing/reducing *Campylobacter* contamination and in controlling the contamination and/or the *Campylobacter* counts on the carcasses.

No association between the *Campylobacter*-contamination result on the broiler carcass and the production capacity of the slaughter-house was observed in the baseline survey. Further potential slaughter-house-specific factors (e.g. slaughter process and technology, hygiene implementation, etc.) of relevance to the *Campylobacter* contamination of broiler carcasses were not part of the EU baseline survey.

Apart from technology-, i.e. process hygiene-related factors, observed heterogeneity between slaughter-houses could be related to the within-batch *Campylobacter* prevalence in the (incoming) slaughter batches or to the bacterial load of the broiler caeca (in addition to the recorded positive-negative status). Slaughter-house-specific effects on *Campylobacter* colonization status of the incoming birds is considered minimal although it may happen when *Campylobacter*-contaminated crates are used for the transport of the flock to the slaughter-house (Hansson *et al.*, 2005). The heterogeneity within slaughter-houses thus to some extent may reflect different occurrence of *Campylobacter*-colonized flocks that are delivered to the slaughter-houses. One possible explanation for differences between slaughter-houses could, therefore, be the tendency for *Campylobacter*-colonized slaughter batches to be sent to certain slaughter-houses. Another explanation could be that certain segments of the broiler slaughter-houses – having their own farms and slaughter-houses – are more conscious about biosecurity and other risk factors at primary production, and place a bigger emphasis on this also at farm level.

Furthermore, even though a colonized broiler batch was more likely to yield a contaminated carcass, in the analysis of the results of the EU baseline survey there were many contaminated carcasses derived from broiler batches that tested negative. Some of these may be due to limitations of the experimental set-up used, and limited testing sensitivity to detect all *Campylobacter*-colonized batches, while others may result from cross-contamination from other carcasses or equipment within the slaughter-houses. Slaughter-house effects might relate to slaughter hygiene practices impacting on the extent to which caecal and faecal contents contaminate carcasses. In analysis of the risk factors for

Campylobacter contamination of broiler carcasses at slaughter-houses in France, it was shown that cross-contamination occurred during the slaughter process as the prevalence of *Campylobacter* was higher on carcasses than in caeca. The number of carcasses on a trolley during chilling tended to be associated with the increase of contamination on carcasses (Hue *et al.*, 2010). *Campylobacter* cross-contamination can occur throughout the entire slaughter process, including the chilling room (Berndtson *et al.*, 1996). Presence of contaminated equipment, work surfaces, process water and air increases the probability for contamination of initially *Campylobacter*-free carcasses. Surfaces may vary in sensitivity to bacterial attachment and biofilm formation. Attachment to, for example, plucker-finger rubber was significantly lower than attachment to stainless steel and other surfaces (Arnold and Silvers, 2000). In particular, aerosols of *Campylobacter* are formed during defeathering (plucking) (Allen *et al.*, 2003; Haas *et al.*, 2005; Johnsen *et al.*, 2007) and cause significant carcass contamination. Evisceration is also a source of carcass contamination and cross contamination (Corry and Atabay, 2001). After processing and chilling the carcasses are sorted, allowing again for cross-contamination with *Campylobacter*. Portioning is usually done mechanically, with more possibilities for cross-contamination among carcasses via the machinery. Some portions, particularly breast fillets, have the skin removed, which usually reduces the numbers of *Campylobacter*.

Thus, GMP/GHP priniciples and process hygiene measures at abattoir level should be optimized, implemented on a daily basis and verified before additional interventions at the abattoir are considered. These intervention strategies are not a substitute for but an addition to the basic GMP and HACCP principles.

4.5.3. Slaughter time

Analysis of risk factors in the EU baseline survey (EFSA, 2010b) showed that the prevalence of *Campylobacter*-contaminated broiler carcasses increased when sampling later during the day. A possible explanation could be cross-contamination from *Campylobacter*-colonized batches slaughtered earlier on the same day (Johannessen *et al.*, 2007). This is consistent with environmental accumulation of contamination. *Campylobacter* can even survive overnight on surfaces of slaughter-house equipment despite of cleaning and sanitizing (Johnsen *et al.*, 2007; Peyrat *et al.*, 2008).

4.6. Interventions against *Campylobacter* during slaughter, dressing and processing

The poultry meat industry starting point is the slaughter-house which, according to the results of the 2008 EU baseline survey (EFSA, 2010b), in most EU countries is highly likely to have *Campylobacter*-positive broilers as starting material. This puts increased pressure on their food safety management system which is primarily based on hygienic measures (GMP/GHP) and implementation of the compulsory HACCP system. However, there are several options under investigation to act as an intervention step in the slaughter or poultry processing line to significantly reduce the numbers of *Campylobacter* spp. on poultry meat put on the market. Table 2 provides a summary of the quantitative effects of interventions on prevalence and numbers of *Campylobacter* at their point of application in the food chain. Table 3: provides information on advantages and disadvantages as well as the availability of these interventions. The expected reduction in public health risk of implementing these interventions is discussed in Chapter 5, based on a Quantitative Microbial Risk Assessment (QMRA).

The efficient implementation of hygiene measures, HACCP or in a generic manner, a Food Safety Management system may lead to low numbers of microorganisms and small variability in microbial counts (Jacxsens *et al.*, 2009). The stringent implementation of HACCP in poultry meat plants may result in significant reductions in the numbers of *Campylobacter* in poultry meat. Federal HACCP regulations required in US to reduce levels of *Salmonella* prevalence appear also to have led to a reduction in *Campylobacter* on processed poultry products in northeast Georgia (Stern and Robach, 2003). In a Belgian study it was also demonstrated that the difference in distribution in *Campylobacter* spp. numbers found in poultry meat in two poultry meat processing companies was also reflected in a difference of their overall microbial performance (as shown by indicator organisms *Enterobacteriaceae* and *E.coli*). Indications were obtained that *Campylobacter* spp. could be dispersed

during processing due to lack of process control and effective GHP, resulting in high prevalence and high counts (Sampers *et al.*, 2010).

4.6.1. Prevention of spillage of intestinal contents

Several studies show that differences in prevalence of *Campylobacter* result from different slaughtering practices (EFSA, 2005; Rosenquist *et al.*, 2006). A significant impact of the different process operations, such as scalding, defeathering, evisceration, washing and chilling on the prevalence of *Campylobacter*-contaminated broiler carcasses was evidenced.

Various studies have demonstrated or confirmed that hygienic design of equipment is important for minimising contamination and growth of pathogens (Luning *et al.*, 2008). Although various studies have confirmed the importance of hygienic design of machinery in food processing, poultry processing machinery has not always been designed with hygiene as a priority. In the slaughter process, contamination may in particular occur during the evisceration operation especially if the machines used for evisceration are not adapted to the natural variation of carcass sizes within a given batch. Consequently, the rupture of viscera may occur and the release of intestinal contents can contaminate the carcasses eviscerated (Figueroa *et al.*, 2009). Berrang *et al.* (2004b) demonstrated that intestinal contents may contaminate broiler carcasses during processing with faecal *Enterobacteriaceae* and zoonotic pathogens such as *Campylobacter*. When analyzing the performance of the food safety management system in two poultry processing companies, the lack of good operational performance of the evisceration step in one of the two companies was shown as one of the factors that contributed to increased numbers and prevalence of *Campylobacter* on the poultry meat (Sampers *et al.*, 2010). Visceral rupture resulted in an increase of 0.9 $\log_{10}$ CFU of *Campylobacter* per carcass, suggesting that *Campylobacter* counts may also be reduced by optimizing the hygienic design of equipment or by physical removal of faecal contamination (Boysen and Rosenquist, 2009). The latter study provides quantitative information in order to study via risk assessment models the effect of prevention of faecal leakage as an intervention step to reduce *Campylobacter* numbers on the broiler carcasses and thus the risk for campylobacteriosis due to poultry consumption.

4.6.2. Scheduled slaughter

Scheduled slaughter means identifying flocks positive for *Campylobacter* spp. before they are slaughtered, and subjecting carcasses from these flocks to special treatment like freezing, heat treatment or other *Campylobacter* reducing measures. This scheduling is routinely used in some countries (Norway, Iceland, and Denmark).

To be able to use scheduled slaughter, the flocks must be sampled before slaughter, so the results from the testing are ready before the slaughter process starts, preferably before the transport to the slaughter-house. With the development of rapid and simple tests, testing can be done a short time before slaughter. This is important, as even many flocks become positive within the last few days before slaughter. In Norway, with an average slaughter age of approximately 32 days, by moving the pre-slaughter sampling from one week before slaughter to four days before slaughter, the percentage of positive flocks which were detected by the pre-slaughter test increased from 50% to 75% (Hofshagen *et al.*, 2010).

4.6.3. Logistic slaughter

Logistic slaughter means slaughtering positive flocks after negative flocks to avoid cross contamination from the positive to negative flocks. Many slaughter houses today perform logistic slaughter based on samples investigated for *Salmonella*. These samples are taken two to three weeks before slaughter, much too early to be of any use regarding *Campylobacter* status of the flock. Additionally, there could be a conflict between logistic slaughter with respect to *Salmonella* and with respect to *Campylobacter* status.

Studies have shown that a rather limited number of carcasses in a negative flock were contaminated by a positive flock slaughtered just before the negative flock. Also, the contaminated carcasses in an

originally negative flock had a low concentration of *Campylobacter* (Hermosilla, 2004; Johannessen *et al.*, 2007). The limited effect of logistic slaughter on the number of human cases is also shown by modelling (Havelaar *et al.*, 2007).

Scheduled and logistic slaughter are difficult to combine in an optimal way, because for logistic slaughter the decision about catching and transportation needs to be made several days before slaughter, which requires early tests, and will result on many flocks becoming positive after the testing has been done, while for scheduled slaughter the samples should be taken as close to slaughter as possible.

Logistic slaughter is therefore not included as an intervention in CAMO.

4.6.4. Decontamination

Decontamination aims to reduce both the prevalence and the numbers of microbes on carcasses. Decontamination may be achieved by physical treatment or by applying a chemical substance to carcasses during the slaughter process. Decontamination should be considered a supplement and not a substitute to good hygiene practices. EC Regulation No 853/2004 allows decontamination treatments to be considered if a substance is shown to be safe and effective. However, no chemical decontamination treatments are currently authorized in the EU but some chemicals are used in a number of other countries worldwide. Physical treatments like freezing and heat treatment, however, are applied in some countries in Northern Europe (Georgsson *et al.*, 2006a; Hofshagen and Kruse, 2005; Rosenquist *et al.*, 2009).

Contamination of carcasses/portions of meat happens during slaughter and further processing, therefore almost all microbial contaminants are located on the skin or on the surface of carcasses or portions. The appearance of the product should be unaffected, so potential treatments affect only the surface, the exception being irradiation.

The following sections describe the effect of various physical and chemical decontamination treatments on numbers of *Campylobacter*. The body of evidence of the studies has been evaluated by the ad-hoc Working Group and the BIOHAZ Panel, taking into account whether the studies were done in the laboratory or slaughter-house, and whether they used inoculated or naturally contaminated chicken. Only studies fulfilling the following criteria were accepted:

- Those that used chicken meat or skin as medium for *Campylobacter* (not laboratory media);
- Those with valid control samples (i.e. untreated samples for physical methods and potable water instead of chemical solutions for the chemical methods);
- Studies where the experimental parameters were properly defined (e.g. with indication of ratio of treatment fluid to weight of chicken, time of application).
- Studies carried out in the slaughter-house using naturally-contaminated carcasses straight from the line were preferred to those using previously processed inoculated carcasses.

Table 1 summarizes the weight given to naturally contaminated versus inoculated chicken and industrial- versus laboratory-scale studies. The criteria were developed in the FAO/WHO report on Benefits and Risks of the Use of Chlorine-containing Disinfectants in Food Production and Food Processing (FAO/WHO, 2008).
The efficacy of the test methods used in the various studies was not evaluated, nor whether these detected sublethally damaged *Campylobacter*, so we merely quote their reported reductions.

Table 1: Relative strength of the contribution of study data to the general body of evidence, based on study type

	Natural contamination	Inoculated studies
Industrial data	High[a]	Not applicable
Pilot-scale data[b]	High[c]	Medium[d]
Laboratory data	Medium[d]	Low[e]

a Ideal studies also quantify counts and prevalence of pathogens with statistical analysis.
b Experiments using industrial equipment in non-industrial settings.
c If the pilot process is representative of the industrial process; otherwise, evidence makes a "medium" contribution to the body of evidence.
d Data would not be sufficient to inform a quantitative microbial risk assessment or to allow definitive conclusions on risk reduction.
e Data are indicative of a disinfectant effect that may be reproducible in practice, but on their own do not allow definitive conclusions on risk reduction.

The effect of various chemical and physical treatments are summarized in Table 2. Included in this table are the data which have been judged the most reliable based on the weighting described above.

4.6.4.1. Chemical decontamination

The following substances have been evaluated: organic acids (lactic and acetic acid), chlorine, aqueous chlorine dioxide, acidic electrolysed oxidising water, acidified sodium chlorite, peracetic acid or trisodium phosphate.

At present none of these chemicals is authorised for use in the EU for decontaminating raw poultry. Lactic acid, chlorine dioxide, acidified sodium chlorite, trisodium phosphate and peroxyacids have been selected as being likely to give rise to negligible toxic residues (EFSA, 2006; SCVPH, 2003). However, up to now, insufficient proof of effect has been submitted to EFSA to justify approval of any chemical. Another aspect is that for some chemical decontaminants, the beneficial effect increases during chilled storage (shelf-life). Few studies have investigated this aspect (Slavik *et al.*, 1994). Neither has the effect on shelf-life of rinsing off the chemical been studied. The limited information available indicates that rinsing is likely to reduce the effectiveness of chemicals. Thus if the EU required that the chemical was washed off shortly after application, much of the benefit is likely to be lost. This prohibition excludes chemical compounds at levels allowed in potable water, which are permitted for use during poultry processing.

The limited information available indicates that rinsing is likely to reduce the effectiveness of chemicals. Most of these chemicals have been investigated in laboratory studies by inoculating samples of skin, meat or whole carcasses, and then dipping them into solutions of the chemicals. Immersion is a very effective method of ensuring full coverage of a product. There are, however, a number of practical problems with immersion. In particular, maintaining chemical concentration is difficult. The activity of the solution will be lost through spillage, absorption by the meat and neutralisation by other organic matter. Acid solutions lose activity as the anions are easily bound by peptides and proteins released by the meat (Smulders, 1995). Chlorine also reacts with organic material (Thomson *et al.*, 1979). Ozone and hydrogen peroxide decompose rapidly in solution (Sofos and Busta, 1992). Extended treatment times can be achieved if water-chilling rather than air-chilling is used, and the chemical compound is added to the water. However, in the EU most poultry is air-chilled, so treatment times involving dips (or sprays) would be short.

Automatic spraying is the alternative method of applying chemicals to chicken carcasses. The effectiveness of automated systems depends upon the influence of various physical parameters. These include nozzle type and configuration, spray pressure, flow rate, and the angle of spray. In addition, variables such as tissue type (skin or meat), natural or artificial contamination, level of contamination, and temperature of treatment all affect the result of washing procedures. A detailed review of spray

washing of meat and produce has been published by Pordesimo *et al.* (2002) where many of the engineering aspects are discussed.

Detailed information on the effectiveness of chemical decontamination to reduce the concentration of *Campylobacter* on chicken carcasses is provided in Appendix B.

While there are many studies using chicken samples inoculated with various bacteria, few published studies have examined the effect of chemicals on numbers of *Campylobacter*s on naturally contaminated carcasses using defined methods (e.g. litres of solution used per carcass, inclusion of water control). Those that have been published show limited effect. We have included data for the following agents in our model (Table 2:): lactic acid, chlorine dioxide, acidified sodium chlorite and trisodium phosphate.

4.6.4.2. Physical decontamination treatments

Physical decontamination treatments are mainly based on treatments which either decrease or increase the temperature of the carcasses or portions or use ionzing radiation. Detailed information on the effectiveness of these methods to reduce the concentration of *Campylobacter* on chicken carcasses is provided in Appendix B.

The most effective methods that should completely eliminate *Campylobacter* from carcasses (assuming no post-process recontamination) are cooking on an industrial scale or irradiation. Cooking clearly changes completely the appearance of the meat from the raw state. Irradiation leaves the meat essentially unchanged in appearance and uses gamma rays from isotopes such as cobalt^{60}, or x-rays or electrons with appropriate energy spectra. Gamma rays and x-rays are more penetrating, and could be used to treat whole carcasses, while electrons are less penetrating, and so would most easily be used on portions. An advantage of x-rays or electrons is that they can be generated using relatively inexpensive machines that can be switched on and off as required, and installed in most slaughter-houses. Another advantage of irradiation is that it would inactivate *Campylobacter* within the meat as well as on the outside, and it could be used on prepacked and/or frozen or chilled product. Irradiation of prepacked product would prevent post-process recontamination.

Freezing to about -20°C for a few weeks is already used to treat carcasses from *Campylobacter*-colonized flocks in a few countries, and reduces numbers by about 2 $\log_{10}$ cycles with minimal impact on the appearance and quality of the meat, although using this technique would require expanded cold-storage facilities, and the increased cost of frozen storage. The effect on *Campylobacter* within the muscle has not specifically been investigated, but is likely also to be lethal. 'Crust freezing' where the surface of the carcass is temporarily frozen on-line during processing also reduces numbers of *Campylobacter*, but to a lesser degree than freezing the whole carcass, and current technology does not seem to include the whole surface. Neither would it affect *Campylobacter* within the muscle.

Heat treatments other than cooking could be added to the processing line in the slaughter-house. Treatment with steam at atmospheric pressure is an attractive option because it would not produce large volumes of dirty water, although hot water immersion systems could be designed with water and energy conservation. There is also some evidence that the effect of steam is enhanced by simultaneous application of ultrasound. Both steam and hot water treatments reduce numbers of *Campylobacter* by 1.5-2 $\log_{10}$ cycles, but *Campylobacter* within the muscle would not be inactivated. The appearance of carcasses treated by either method is changed to some extent, most important is the tendency for the skin to shrink and become more fragile, and for any exposed muscle to change colour slightly, In addition, the carcasses stiffen up, making 'trussing' more difficult. However, the appearance of portions prepared after treatment of carcasses is almost unaffected.

We have included data for the following agents in our model (Table 2): freezing for a few days or for three weeks, hot water immersion, irradiation, cooking, crust-freezing, steam treatment and steam treatment combined with ultrasound.

4.6.4.3. Advantages, disadvantages and availability of interventions

Table 3 presents considerations on the advantages and disadvantages of the interventions discussed in this Opinion from a scientific and technical point of view. Additional considerations, such as costs and acceptance by consumers or the industry are not considered. The availability of interventions is also indicated in the Table. The information in the Table is based on expert opinion in the ad-hoc Working Group and the BIOHAZ Panel, as there is no published data available.

Table 2: Overall summary of effects of interventions

	Efficacy for *Campylobacter* reduction at the point of application	**Modelled**	**References**
Interventions in primary production			
Hygiene/biosecurity	At 21 days: from 20.0% to 7.7% between-flock prevalence (BFP) At 28 days: from 32.0% to 12.0% BFP At 35 days: from 44.0% to 30.8% BFP At 42 days: from 70.8% to 38.5% BFP Implemented in model as the beta coefficient that corresponds to a hazard ratio of 0.40, (0.15, 1.09) p=0.06	Yes	Gibbens *et al.*, 2001
Fly screens	At 21 days: from 11.4% to 5.8% BFP At 28 days: from 28.6 to 5.8% BFP At 35 days: from 45.5% to 7.7% BFP Implemented in model as a slaughter age-weighted k-factor of 0.47 (21 days of slaughter age), 0.15 (28 days of slaughter age) and 0.10 (35 days of slaughter age)	Yes	Hald *et al.*, 2007
Discontinued thinning	BFP estimate OR = 1.74, implemented in model as regression coefficient (0.5521)	Yes	EFSA, 2010a
Slaughter age	BFP estimate OR = 1.98 per 10 days increase, implemented in model as regression coefficient (0.06742)	Yes	EFSA, 2010a
Vaccination	2 $\log_{10}$ reduction in caecal contents	No	de Zoete *et al.*, 2007
Bacteriocins	5.1-5.9 $\log_{10}$ reduction in caecal contents	No	Svetoch *et al.*, 2008
Bacteriophages	3 $\log_{10}$ reduction in caecal contents	No	Wagenaar *et al.*, 2005
Drinking water treatment with organic acids	0.5-2 $\log_{10}$ reduction in caecal contents	No	Chaveerach *et al.*, 2004
Feed additives	No effect to complete inhibition	No	Hilmarsson *et al.*, 2006 Solis de los Santos *et al.*, 2010 Skanseng *et al.*, 2010

	Efficacy for *Campylobacter* reduction at the point of application	Modelled	References
Interventions during transport and before slaughter			
Feed withdrawal	Various results and various outcomes	No	
Crate treatment	7.5 $\log_{10}$ per crate compartment; 5.5 log per crate surface; 40-60% reduction of crate positivity	No	Berrang *et al.*, 2004a Allen *et al.*, 2008a Slader *et al.*, 2002
Interventions at slaughter			
Prevention of leakage of intestinal contents	0.9 $\log_{10}$ CFU reduction on carcass	No	Boysen and Rosenquist, 2009
Detection/re-processing of highly (faecally)-contaminated carcasses	1.75 $\log_{10}$ CFU on carcass	No	Kemp *et al.*, 2001
Cloacal plugging	0.53-1.7 $\log_{10}$ CFU reduction	No	Musgrove *et al.*, 1997 Berrang *et al.*, 2001 Buhr *et al.*, 2003
Scheduled slaughter (positive batches are scheduled to a risk reducing procedure such as freezing or heat treatment)	Depends on risk reducing procedure	Yes (not directly in model, but included by using baseline results and assuming a 100% effective treatment on scheduled batches	Hofshagen et al., 2008. EFSA, 2010a
Logistic slaughter (the slaughter of negative batches before the positive)	Very little effect.	No	Havelaar *et al.*, 2007
Interventions post slaughter			
Chemical decontamination of carcasses			
Lactic acid (2%)	0.47 $\log_{10}$ reduction (through inside-outside bird washer (IOBW)	Yes	Bolder, 2007
	0.74 $\log_{10}$ reduction (inoculated skin)		Riedel *et al.*, 2009
Acidified sodium chlorite (1200 mg/l)	1.26 -1.75 $\log_{10}$ reduction (sprayed after IOBW)	Yes	Bashor *et al.*, 2004
	1.75 $\log_{10}$ reduction (sprayed after IOBW)		Kemp *et al.*, 2001
	0.5 $\log_{10}$ cycles (in IOBW)		Bolder, 2007
	0.5 -1 $\log_{10}$ when sprayed at 1000 ppm		Corry *et al.*, 2008

	Efficacy for *Campylobacter* reduction at the point of application	**Modelled**	**References**
Chlorine dioxide	0.49 $\log_{10}$ reduction (4.25 ppm in IOBW)	No	Bolder, 2007
(50-100 mg/l)	0.99 -1.21 $\log_{10}$ reduction (50 or 100 ppm, dip – inoculated)		Hong *et al.*, 2008
Trisodium phosphate	1.03 $\log_{10}$ reduction (spray)	Yes	Bashor *et al.*, 2004
(10-12%, pH 12)	1.2 $\log_{10}$ reduction (dipping at 50°C)		Slavik *et al.*, 1994
	No effect of dipping at 20°C		Whyte *et al.*, 2001b
	0.5 $\log_{10}$ when sprayed at 12%		Corry *et al.*, 2008
Acidified electrolysed oxidising water (immersion)	1.07 $\log_{10}$ reduction	No	Kim *et al.*, 2005
Peracetic (peroxyacetic) acid	43% reduction of positive carcases	No	Bauermeister *et al.*, 2008a
Physical decontamination of carcasses			
Freezing for few days	0.91 -1.44 $\log_{10}$ reduction	Yes	Sandberg *et al.*, 2005 Georgsson *et al.*, 2006a Rosenquist *et al.*, 2006
Freezing for 3 weeks	1.77 - 2.18 $\log_{10}$ reduction	Yes	Sandberg *et al.*, 2005 Georgsson *et al.*, 2006a
Hot water immersion	1.25 $\log_{10}$ reduction	Yes	Corry *et al.*, 2006
Irradiation	6 $\log_{10}$ reduction	Yes	Farkas, 1998 or expert opinion
Cooking	6 $\log_{10}$ reduction	Yes	Whyte *et al.*, 2006
Crust-freezing	0.42 $\log_{10}$ reduction	No	Boysen and Rosenquist, 2009
Steam	0.46 $\log_{10}$ reduction	No	Whyte *et al.*, 2003
Steam ultrasound	1.3-2.51 $\log_{10}$ reduction	No	Boysen and Rosenquist, 2009

Table 3: Advantages, disadvantages and availability of interventions

	Advantages in addition to a possible *Campylobacter* reducing effect	**Disadvantages**	**Availability**
Interventions in primary production			
Hygiene/biosecurity	Excludes other infectious (animal) diseases as well, some of economic importance. Reduces environmental contamination and indirect transmission to humans.	Complex mixture of factors, difficult to define and audit. Very stringent implementation needed. Farmer compliance required. Only fully applicable to indoor rearing	Immediately available, but might need modification of poultry houses. General principles are well known but needs to be evaluated under local conditions. Only one intervention experiment in UK available
Fly screens	Reminds the farmers of need for hygiene. Effective against seasonal peak in birds. Reduces environmental contamination and indirect transmission to humans.	Only fully applicable to indoor rearing. Applicability depends on construction of poultry houses. Needs maintenance for keeping efficiency.	Rapidly available in theory. Only tested in Denmark and Iceland.
Discontinued thinning	Avoids stress at thinning. Increased animal welfare.	Interferes with current industrial practices. Productivity and flexibility of industrial production will be altered.	Immediately available, in theory.
Reduction of slaughter age	Potentially increased animal welfare.	Interferes with current industrial practices. Productivity and flexibility of industrial production will be altered. For the organic and traditional free range chickens, the slaughter age must not be lower than 81 days.	Immediately available, in theory
Vaccination	Applicable to both indoor and outdoor rearing Multiple vaccines are often applied at same time and systems for the mass application of vaccines are available.	Most studies have been poorly reproducible.	Vaccines are still in the development phase.

	Advantages in addition to a possible *Campylobacter* reducing effect	**Disadvantages**	**Availability**
Bacteriocins	Applicable to both indoor and outdoor rearing.	Scale-up of bacteriocin production and purification remains to be further elaborated.	Preparations have been described, and patents have been applied for.
		Small-scale studies from only one research group, its reproducibility remains to be confirmed.	Not yet tested on large scale.
		Sustainability to be confirmed and take into account the variety of *Campylobacter* species, genotypes and the species' genetic variability.	
		Safety aspects for use to be confirmed.	
Bacteriophages	Applicable to both indoor and outdoor rearing.	Emergence of phage-resistent *Campylobacter* strains needs to be further evaluated under field conditions.	Only tested in small scale experiments.
		Multiple phage populations will be required taking into account the variety of *Campylobacter* species, genotypes and the species' genetic variability.	
		Sustainability to be confirmed.	
Drinking water treatment with organic acids		Biofilms on drinkers may be a challenge.	Conflicting evidence on effectiveness.
		Low pH to control biofilm build-up could lead to welfare issues	Not yet tested on large scale.
		Palatability for birds.	
Feed additives		In some studies a reduced growth rate was observed.	Not yet tested on large scale.
Interventions during transport and before slaughter			
Feed withdrawal	Current guidelines based on animal welfare considerations appear to be optimal for control of *Campylobacter* contamination as well.	Inadequate available data, complex variables and confounding factors involved make it difficult to assess any beneficial effect of feed withdrawal or good hygiene practices during transportation and holding before slaughter. Not yet tested on a large scale.	Immediately available.
Crate treatment	Limits spreading of faeces.	Inadequate available data, complex variables and confounding factors involved make it difficult to assess any beneficial effect of crate treatment.	Not yet tested on a large scale.

	Advantages in addition to a possible *Campylobacter* reducing effect	**Disadvantages**	**Availability**
Interventions at slaughter			
Prevention of faecal leakage	Can be applied to colonized flocks.	Interferes with current industrial practices using high-throughput slaughtering and processing lines. Effect post-chill needs to be investigated.	Equipment not commercially available.
Detection/re-processing of highly faecal-contaminated carcasses	Eliminates high level contaminated carcasses.	Effect on-line has not been demonstrated.	Immediately available.
Cloacal plugging	Can be applied to colonized flocks.	Complex methodology.	Equipment not commercially available.
Scheduled slaughter (positive batches are scheduled to a risk reducing procedure such as freezing or heat treatment)	Reduces the number of flocks to be subjected to further treatment, if considered.	Particularly effective in low prevalence countries Need of reliable and sensitive testing methods for *Campylobacter* spp.	Immediately available. No internationally standardized PCR-method available.
Logistic slaughter (the slaughter of negative batches before the positive)		Impractical if high between-flock prevalence. Need of reliable and sensitive testing methods for *Campylobacter* spp. Testing must be done as close to slaughter as possible May also need to consider *Salmonella* carriage. Not effective for public health as numbers of *Campylobacter*s on negative batches processed after positive ones are very low.	Immediately available.
Interventions post slaughter			
Chemical decontamination of carcasses			
All chemicals		Risk of residues and by-products. Issues of waste water management.	Available in the short term. Currently no chemicals are approved in the EU.

	Advantages in addition to a possible *Campylobacter* reducing effect	**Disadvantages**	**Availability**
Lactic acid	Occurs naturally in meat. No organoleptic effect when used at low concentrations, e.g. 2%	Carcass discoloration might occur at high concentrations. 2% lactic acid would not significantly affect carcass colour.	Available in the short term. Currently not approved in the EU.
Acidified sodium chlorite	Effective as a dip or spray.	Unpleasant for operatives. Has to be prepared on-site.	Available in the short term. Currently not approved in the EU.
Chlorine dioxide	Better effect can be expected post-washing.	Conflicting results. Unstable and has to be prepared on-site. Effect will depend on presence of organic substances.	Available in the short term. Currently not approved in the EU.
Trisodium phosphate	Effective as a dip or spray.	Negative environmental impact of phosphates. Unpleasant for operatives.	Available in the medium term.. Currently not approved in the EU.
Acidified electrolysed oxidising water (immersion)	Could be used during water chilling.	Not tested on-line or on naturally contaminated carcases.	Available in the short term.. Currently not approved in the EU.
Peracetic (peroxyacetic) acid		Not tested on line or on naturally contaminated carcasses.	Available in the short term.. Currently not approved in the EU.
Physical decontamination of carcasses			
All physical treatments	No residues.	Energy consuming.	Can be used in without specific authorisation all EU countries (except irradiation).
Freezing for few days/ 3 weeks	Proven on production scale. Effective and implemented in some countries.	Thawing causes drip, which may cause cross-contamination.	Available in the short term.
Hot water immersion	Product still fresh.	Reduced product quality (appearance affected in some studies). No on-line equipment available.	Available in the medium term.
Irradiation	Product still fresh. Eliminates *Campylobacter*s inside the muscle and liver.	Not feasible for whole carcasses unless x-rays or gamma radiation from isotopes used.	Available in the medium term. Not authorised for use in all EU countries

	Advantages in addition to a possible *Campylobacter* reducing effect	**Disadvantages**	**Availability**
Cooking	No residues.	Not fresh meat anymore.	Immediately available, in theory.
		May only be possible to apply to a small proportion of products.	
		Variability in survival depending upon the product, the strain and the procedure for heat treatment (pan-frying, oven heating etc).	
		May not be popular with consumers.	
Crust-freezing	Product still fresh.	Only proven on-line for breast fillets, not feasible for whole carcasses.	Available in the short term.
Steam	Product still fresh.	Reduced product quality (appearance affected in some studies).	Available in the medium term.
	In-line equipment could be designed and installed easily on existing lines.	Slight shrinkage of skin which becomes less pronounced after storage.	
	No issue with waste disposal	No on-line equipment available.	
Steam ultrasound	No residues.	Slightly boiled appearance of skin using proof-of-concept apparatus (highest efficacy).	Available in the short term.
	Product still fresh.	Product quality maintained using on-line equipment (lower efficacy).	

5. Public Health impact of controlling *Campylobacter* in the broiler meat production chain

5.1. Review of published risk assessments on control options

A number of quantitative microbiological risk assessments (QMRAs) on *Campylobacter* in broiler meat have been performed in Europe and elsewhere (for a review see Nauta *et al.* (2009a)). Depending on the objectives of these risk assessments, they were used to evaluate different sets of control options in the broiler meat production chain. In principle, control options can be evaluated in a generic way and for specific interventions. In the generic approach a hypothetical decrease in prevalence or concentration at a specified point in the production chain is evaluated for human risk reduction at the end of the food chain, where the risk reduction is the predicted human incidence after intervention, compared to the human incidence in the original situation (the baseline). If specific interventions are evaluated, the effect of a control option or intervention measure is interpreted as a change in prevalence or concentration at the point of intervention, and the QMRA model is used to translate this into a risk reduction.

Many studies have found (an approximately) linear relationship between flock prevalence and human health risk (Nauta *et al.*, 2009a; Rosenquist *et al.*, 2003), which implies that a certain percentage of reduction in flock prevalence results in a similar proportion of risk reduction. However, the same relationship cannot be observed when the concentration of *Campylobacter* is taken into account. A decrease in levels of contamination of contaminated servings usually results in a non-linear decrease of the predicted incidence of infection. This depends mainly on the non-linear effect of cross-contamination simulated in the consumer phase of the QMRA model and the dose-response model applied for the estimation of the final impact on human health (Nauta *et al.*, 2009a).

The QMRA models developed to date consider the prevalence of contaminated slaughter batches and the concentration of *Campylobacter* on the carcasses as two independent variables. It is reasonable, however, to suppose a relationship between these two values, as partially confirmed by the analysis of the results of the EU baseline survey on prevalence of *Campylobacter* in broiler batches and of *Campylobacter* and *Salmonella* on broiler carcasses performed in the EU in 2008 (EFSA, 2010a).

In Table 4 the predicted risk reductions found in various generic approaches (Brynestad *et al.*, 2008; FAO/WHO, 2009b; Lake *et al.*, 2007; Lindqvist and Lindblad, 2008; Rosenquist *et al.*, 2003) are compared with the results of the evaluation of specific interventions found by Nauta (2005a), Havelaar *et al.* (2007) and Gellynck *et al.* (2008). These results confirm that one or two $\log_{10}$ reductions in concentration on chicken carcasses can significantly reduce human health risks, when all other risk factors are constant.

In addition, the effect of decrease in concentration differs depending on: (1) the stage of broiler meat production at which the intervention is applied and (2) whether *Campylobacter* is located in the intestinal contents, on the bird's exterior or on the carcass. For example, a $\log_{10}$ reduction in concentration at scalding has a less pronounced effect on the final numbers of *Campylobacter* on the carcass than a $\log_{10}$ reduction after chilling (Table 4). However, it is important to highlight that a comparison of risk reductions from different risk assessment studies can only be used for a quick glance on the different predicted effects of relative risk reductions consequential to interventions during industrial processing. Each study, in fact, should be considered in relation to its specific assumptions, input variables considered and mathematical/statistical approaches used. The results of different risk assessment studies, therefore, might be not fully comparable to each other.

In general, different QMRAs found that logistic slaughter, the separate slaughter, dressing and processing of negative and positive flocks, would have a negligible effect on human health risk (see 4.6.3)(Havelaar *et al.*, 2007; Rosenquist *et al.*, 2003). It is also concluded that reducing the level of *Campylobacter* at carcass level is an efficient intervention method. Currently, the most efficient intervention measures at carcass level aim at reducing rather than eliminating *Campylobacter* from the carcasses (Nauta *et al.*, 2005b), because levels of contamination are usually too high to be eliminated.

The exceptions to this would be irradiation and cooking. The public health benefits of reducing *Campylobacter* numbers by 2-3 $\log_{10}$ units are expected to be considerable.

Another general finding is that it is difficult to obtain good representative data that allow estimating the effect of specific control options in terms of reduction in *Campylobacter* concentration or prevalence. Quite often the effect estimates are based on one or a few published or unpublished laboratory experiments, or expert opinion, and they cannot always be correctly applied to conditions other than the specific ones under which they were designed. As a consequence their predicted effects on risk reduction are also highly uncertain (see Chapter 4).

Table 4: Examples of reported risk reductions as a consequence of reduction on *Campylobacter* concentrations due to the application of control options along the broiler meat processing chain.

Reference of QMRA	Point of the chain	Target parameter	Effect ($\log_{10}$ reduction)	Risk reduction (% of human incidence)
Rosenquist *et al.* (2003) - generic reduction of concentration on carcasses			-2	97%
Lake *et al.* (2007) - generic reduction of concentration on carcasses			-1 -2	71% 88%
Brynestad *et al.* (2008) - generic reduction of concentration on carcasses	Processing plant	$\log_{10}$ number (CFU) of *Campylobacter* on carcass	-0.2	30%
Linqvist and Lindblad (2008) - generic reduction of concentration on carcasses			-2	92% – 97%[#]
FAO/WHO (2009b) - generic reduction of concentration on carcasses			-0.25	11% – 82%[*]
Nauta *et al.* (2005b) and **Havelaar *et al.* (2007)**[§]				
Phage therapy	Farm	$\log_{10}$ number (CFU) of *Campylobacter* in faeces	-1 / -2 / -2	74.4%
Reduction of faecal leakage	Processing plant	$\log_{10}$ number (CFU) of *Campylobacter* in faeces	0 / -6 / -∞	77.1%
Decontamination in the scalding tank: - by adding lactate - by adding TSP (trisodium phosphate)	Processing plant	$\log_{10}$ number (CFU) of *Campylobacter* on carcass	-0.3 / -0.8 / -2 -1.03 / -1.24 / -1.5	12.4% 18%

Reference of QMRA	Point of the chain	Target parameter	Effect (log_{10} reduction)	Risk reduction (% of human incidence)
Decontamination before chilling: - using lactic acid - using TSP (trisodium phosphate)	Processing plant	Log_{10} number (CFU) of *Campylobacter* on carcass	-0.3 / -1.3 / -2 -1.03 / -1.24 / -1.5	86.9% 90.6%
Other decontamination measures: - only dipping - dipping and spraying - crust freezing - irradiation - freezing of products	Processing plant	Log_{10} number (CFU) of *Campylobacter* on carcass	-0.3 / -1.3 / -2 3*(-0.27 / -0.6 / -0.83) -0.4 / -1.1 / -1.7 -4.7 / -10.5 / -20.8 -0.9 / -1.7 / -3.2	77% 80% 82.8% 100% 94.9%
Gellynck *et al.* (2008): [§]				
Phage therapy	Farm	Log_{10} number (CFU) of *Campylobacter* in faeces	-1 / -2 / -3 (-1 on external)	53% – 76% - 82%
Carcass decontamination - crust freezing - lactic acid[¥] - electrolyzed oxidizing water[¥] - irradiation	Processing plant	Log_{10} number (CFU) of *Campylobacter* on carcass	-0.4 / -1.1 / -1.7 -0.3 / -1.3 / -2 -1.1 / -2.3 / -3 -4.7 / -10.5 / -20.8	32% – 61% – 82% 0% – 38% – 72% 28% – 80% – 91% 99.8% – 100% – 100%

[#] if fresh or frozen chicken respectively are considered.
[*] depending on the initial concentration equal to 6 log CFU and 2 log CFU respectively.
[§] based on three different levels of efficacy (pessimistic, most likely, optimistic) of each measure. The outcomes are expressed as mean risk reduction values.
[¥] Used to replace carcass washing

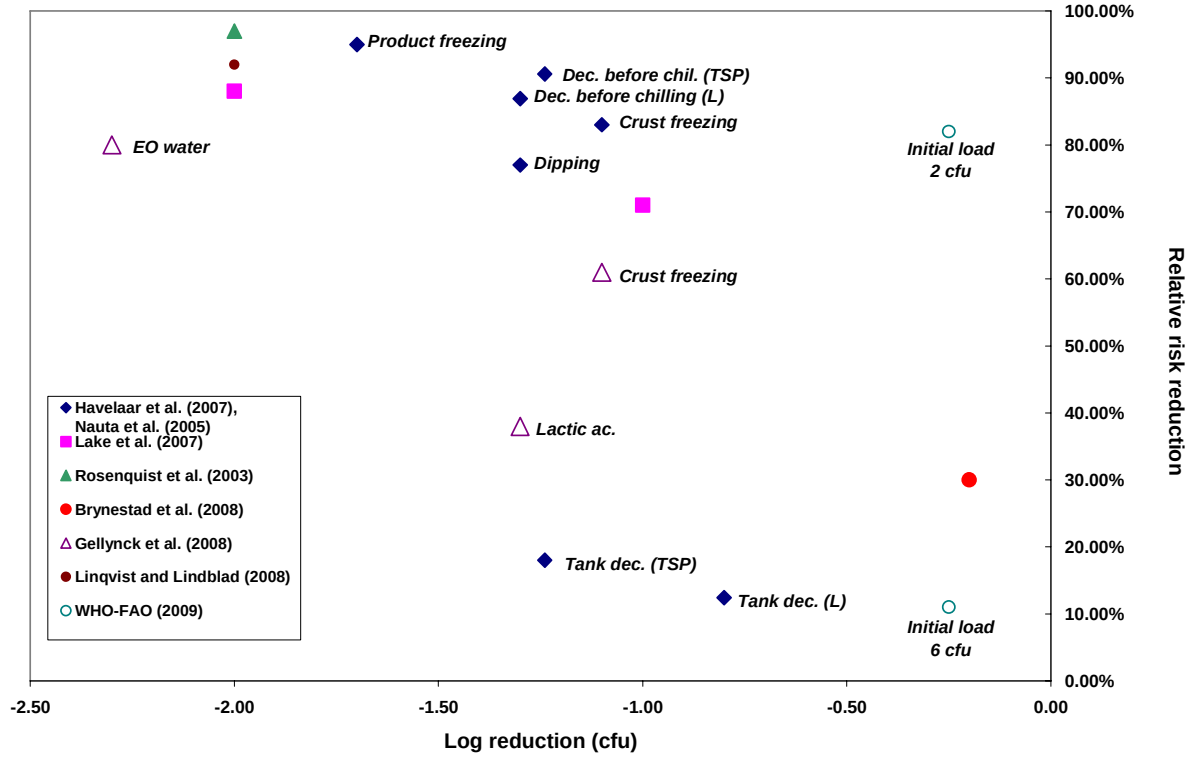


Figure 4: Comparison of reported risk reductions as a consequence of a reduction in concentration on carcasses.

5.2. Quantitative Risk Assessment

The European Food Safety Authority (EFSA) published a call for a "Quantitative Microbiological Risk Assessment of *Campylobacter* in the broiler meat chain" and a contract was awarded to develop a full farm-to-consumption QMRA. The contractor provided a software tool to evaluate interventions. The commissioned model (CAMO) uses many of the same principles of previous food safety risk assessment models, but takes a different mathematical approach to achieve its results. In previous risk assessment models, the variability of the level of contamination in different steps of the food chain is described by some parametric distribution (e.g. lognormal), and effects of processing, interventions etc. are evaluated using Monte Carlo simulation. CAMO characterizes the variability of the level of contamination by the normalized central moments (mean, variance, skweness and kurtosis) of the $\log_{10}$ numbers and evaluates the effects of processing, interventions etc. by combining the raw moments of variables in the model using analytical mathematical equations. The full report on the development of the model is published on EFSA's website[13]. A peer review was undertaken as well as an evaluation of the Method of Moments from a statistical point of view.

The focus of CAMO was to evaluate the human health consequences of interventions as specified in Chapter 4 using the following general approach:

1. CAMO was built to reflect the situation in the broiler meat chain for all individual EU MSs, based on data from the EU baseline survey (EFSA, 2010a). The model also needs as an input the extent to which specified interventions were implemented in a country.

2. CAMO was calibrated to observed rates of human campylobacteriosis in the MSs that can be attributed to broiler meat using two different "dose-response" (DR) models ("simple exponential DR model" and "modified Beta-Poisson DR model"). These "dose-response" models relate the

[13] www.efsa.europa.eu/en/supporting/doc/132e.pdf

distribution of the number of *Campylobacter* on contaminated carcasses to the number of cases of human campylobacteriosis attributable to broilers in a MS. All potential steps between the production of chilled carcasses and the exposure of a random person to *Campylobacter* from a contaminated carcass are implicitly encompassed by this model. Hence, both models differ from standard dose-response models as described by FAO/WHO (FAO/WHO, 2003) in which the dose is defined at the point of consumption rather than at some previous step in the food chain.

CAMO uses rates of campylobacteriosis in MSs as reported in the Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union (EFSA, 2010c), corrected for underreporting by multipliers as described in Chapter 1.2 and Appendix A. Attribution of human cases to broiler meat was set at 30% by default based on the EFSA opinion on human campylobacteriosis (EFSA, 2010d). The effect of interventions on microbial load and/or between-flock prevalence and/or within-flock prevalence was implemented in the model and the relative change in the predicted human health burden was evaluated.

3. The effect of interventions on microbial load and/or between-flock prevalence and/or within-flock prevalence was implemented in the model and the relative change in the predicted human health burden was evaluated.

4. CAMO can be run in a deterministic mode (i.e. model parameters are represented by a single best-estimate value) or in stochastic mode (i.e. model parameters are provided as uncertainty distributions, which are evaluated using Monte Carlo simulation).

For evaluation of interventions aimed at reducing *Campylobacter* concentrations, CAMO was modified. Instead of the "dose-response" (DR) models proposed by the contractor, it was decided to use the generally accepted approach to dose-response modelling, i.e. to estimate the risk of infection and/or illness as a function of the ingested dose at the point of consumption (FAO/WHO, 2003). Bacterial counts on broiler meat were provided by a simple processing model, assuming a proportional relationship with counts on carcasses. The relationship between bacterial counts on meat and ingested doses was provided by the combination of a published consumer phase model for *Campylobacter* on chicken meat (Nauta et al 2008) and the commonly applied dose response model for *Campylobacter* (Teunis and Havelaar 2000), as discussed by Nauta and Christensen (2011). This modification of CAMO will be described as the classic+ DR model. Sensitivity analyses on the choice of the DR model were performed. A discussion of the validity of these different modelling approqaches is presented in Chapter 5.3.1. The model was only run in deterministic mode because there was insufficient information to quantify uncertainty in the model parameters.

CAMO could not be used to evaluate scheduled slaughter, nor the public health impact of microbiological criteria. Because the model works only with moments of distributions, and not with a specified distributional form, it is not possible to sample from the distributions, nor to define percentile values at any point in the food chain, which would be necessary for such evaluations.

Additional modelling approaches were developed for these purposes, as described in Chapters 5 and 6.

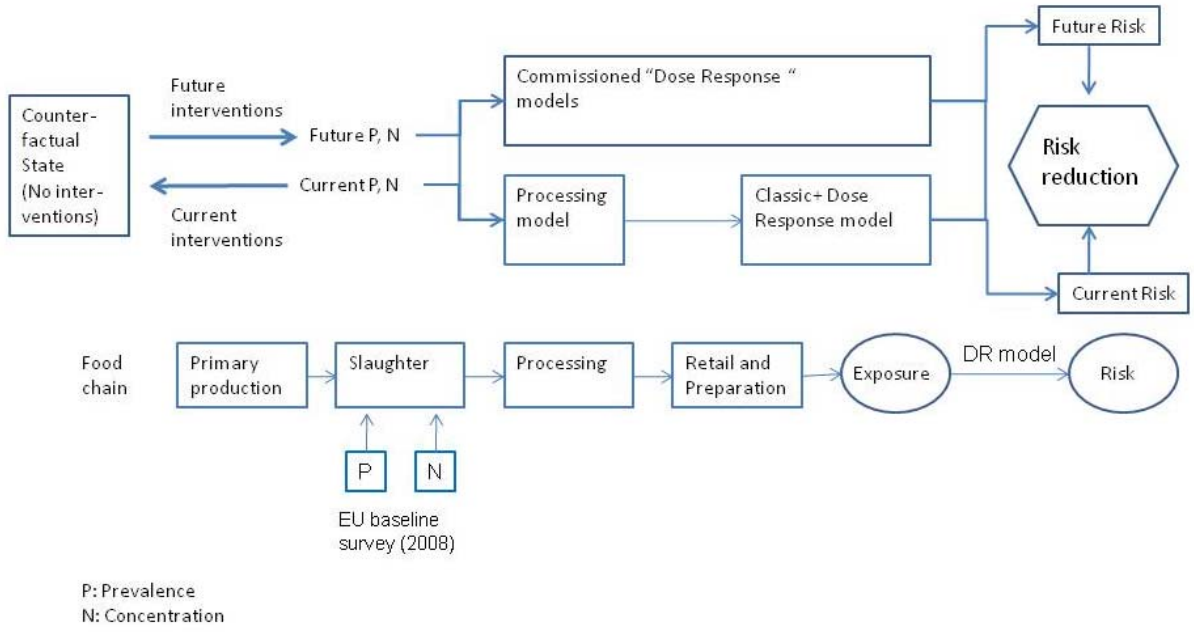


Figure 5: Modelling approach used to estimate risk reductions due to interventions during primary production and slaughtering

5.2.1. Model inputs

The effect of interventions was modelled for four countries based on the prevalence of *Campylobacter*-positive batches, level of carcass contamination as reported in the EU baseline survey and on the availability of data.

Table 5: Selection of countries for modeling based on EU baseline survey data

	Country 1 (C1)	**Country 2 (C2)[a]**	**Country 3 (C3)**	**Country 4 (C4)**
Prevalence of *Campylobacter*-colonized batches	Low	medium	medium	high
Level of carcass contamination	Low	high	medium	medium

[a]C2 has a relatively high proportion of outdoor flocks

To populate the model, the following assumptions were made:

- *Campylobacter* colonization of broiler flocks is common in the EU, hence current levels of biosecurity cannot be assumed to be sufficient to control the entry of *Campylobacter* into the broiler house. The summer peak in flock prevalence is thought to be due to specific seasonal factors such as an increased burden of environmental contamination with organisms as well as reduced effectiveness of hygiene barriers due to the activity of flies, increased ventilation, etc. On this basis it can be presumed that the number of indoor flocks colonized in winter for any MS reflects the maximum efficacy of biosecurity in that country. By extrapolation, the maximum efficacy of biosecurity for each MS was defined as 100% minus the average % winter prevalence in that country (January, February, March), as determined by the EU baseline survey (2008). In contrast, the number of flocks colonized in summer presumably reflects the lowest efficacy of biosecurity. Therefore, this minimum efficacy was defined as 100% minus the average % summer prevalence (July, August, September) for each country. Thus, the most likely value of % efficacy of biosecurity was the mean of these minimums and

maximums for each country. Given previous considerations about biosecurity levels in outdoor flocks, the efficacy of biosecurity with regard to *Campylobacter* in such flocks was considered to be 0%.

- The following interventions were assumed not to be currently used: bacteriophages, bacteriocins, vaccination, fly screens.
- On farm water treatment with organic acids was only assumed to be used in C2 (30%, expert estimate).
- As explained above, the model was not deemed suitable for evaluating scheduled slaughter, so this could not be implemented in the current state. Although scheduled slaughter (with diversion of positively tested flocks to freezing) is practised in C3 and C1, the default value has been set to zero.
- Detection of highly contaminated carcasses and prevention of faecal leakage: no objective measure was available and the model was run in deterministic mode for 50%.
- All decontamination before and after chilling (physical and chemical) was set to 0%.

Table 6: Summary of model inputs (most likely values) used to evaluate intervention scenarios.

Intervention	C1	C2	C3	C4
Farm				
Indoor farms as % of total	100	71	100	94
Mean age at slaughter[1]	32	41	38	40
Thinning as % of all batches[1]	3	82	25	65
Between-flock prevalence[3]	3 (0-8)	30 (24-31)	19 (6-38)	76 (67-86)
Hygiene/biosecurity[1]	96	73	78	24
Bacteriophages	0	0	0	0
Bacteriocins	0	0	0	0
Vaccination	0	0	0	0
Fly screens	0	0	0	0
Water treatment with organic acids[2]	0	30	0	0
Transport				
Any intervention	0	0	0	0
Slaughter				
Campylobacter count[4] on neck/breast skin after chilling (log_{10} CFU/g)	0.94 (0.29)	2.72 (0.85)	2.21 (1.2)	2.36 (1.34)
Logistic slaughter	0	0	0	0
Scheduled slaughter	0	0	0	0
Detection of highly contaminated carcasses	50	50	50	50
Prevention of faecal leakage	0	0	0	0
Decontamination	0	0	0	0
Freezing	0	0	0	0
Reporting factor human cases	42.5%	4.2%	30.0%	11.4%

[1] Indoor farms only
[2] Indoor and outdoor farms
[3] Overall prevalence (winter – summer prevalence)
[4] Mean (standard deviation) from positive batches only

5.2.2. Interventions

The public health impact of interventions described in this section relate to the number of cases of human campylobacteriosis associated with the preparation and consumption of broiler meat. As

explained in section 4.2 interventions in primary production may further reduce numbers of human cases. However this cannot currently be quantified.

5.2.2.1. Hygiene barriers

Only one study was available from the literature that provided a quantitative estimate of the effect of hygiene barriers on full scale farms. This study from the UK (Gibbens *et al.*, 2001), which is discussed in detail in Chapter 4.2.1., was carried out approximately 10 years ago and since then, the UK industrial practice may have changed, as there have been initiatives to improve biosecurity. In particular, in January 2004 a campaign[14] was launched to help improve hygiene measures on broiler farms and ensure that best practices are followed at all times. Improving hygiene barriers in all indoor flocks in the UK (compared to the 24% currently deemed to have effective biosecurity) would reduce the public health risk by 17%, with a 95% confidence interval of between 0 and 39%. As discussed in Chapter 4.2.1.1, applying hygiene barriers is assumed to be ineffective in controlling *Campylobacter* in outdoor flocks, hence the overall effect of this intervention excluding outdoor flocks would be slightly less at 16 (0 – 37)%. If the percentage of outdoor flocks increased in the future, the overall effect might be reduced even more. A linear relationship was observed between the percentage of flocks where hygiene barriers were in place and the public health risk reduction. For example, applying hygiene barriers to 50% of all indoor flocks, compared to the current 24%, would result in a risk reduction of 6% instead of 16% when applied to all indoor flocks. Thus, the efficacy of hygiene barriers, as described by Gibbens *et al.* (2001), is very uncertain. The impact of hygiene barriers was assessed for C4 only.

5.2.2.2. Fly screens

Only one study was available from the literature that provided a quantitative estimate of the effect of fly screen interventions on full scale farms. This study from Denmark (Hald *et al.*, 2007) is discussed in detail in Chapter 4.2.1.2. Applying fly screens to all indoor flocks (compared to no fly screens currently) would reduce the public health risk by 60%. Thus, the effect of fly screens may result in a considerable reduction of public health risk. Obviously, fly screens are not applicable to outdoor flocks, but with a low percentage of outdoor flocks, the overall effect of the intervention is the same. If the percentage of outdoor flocks were to increase in future, the overall effect might be reduced.
The impact of fly screens was assessed for C3 only.

5.2.2.3. Discontinued thinning

Discontinued thinning is discussed as a potential intervention in Chapter 4.2.4 because of the risk of infecting the remainder of the birds with *Campylobacter* brought in by the catching crews and poorly cleaned and disinfected transport crates. Based on the multivariate regression model from the statistical analysis of the baseline study data (hence accounting for effects of the age at slaughter), it was estimated that the effect of discontinued thinning would differ greatly between countries. Stopping thinning completely could reduce the public health risk by only 2% in C1, 13% in C3 and up to 25% in C2 and the C4. The difference between effects in these countries is related to differences in the percentage of flocks currently thinned, and to the fraction of outdoor flocks, which were assumed not to undergo thinning in these countries.

5.2.2.4. Slaughter age

The beween-flock prevalence (BFP) increases with age at slaughter and therefore reducing slaughter age has a theoretical effect on the public health risk depending on the current age at slaughter and the actual BFP. The evaluation of this intervention was only applied to indoor flocks, as poultry raised in outdoor flocks take, on average, longer to grow, and some production systems require outdoor birds to reach a certain age before being marketed. The public health benefit of restricting slaughter age is equivalent to the reduction in BFP. Restricting slaughter age to 35 days resulted in 9-18% risk reduction in three countries but had no effect in C1 (current average slaughter age 32 days).

[14] www.food.gov.uk/safereating/microbiology/flocks/

Restricting the slaughter age to 28 days resulted in a 22-43% risk reduction. Hence, restricting the slaughter age to below five weeks might result in appreciable public health benefits. Results are illustrated in Figure 6.

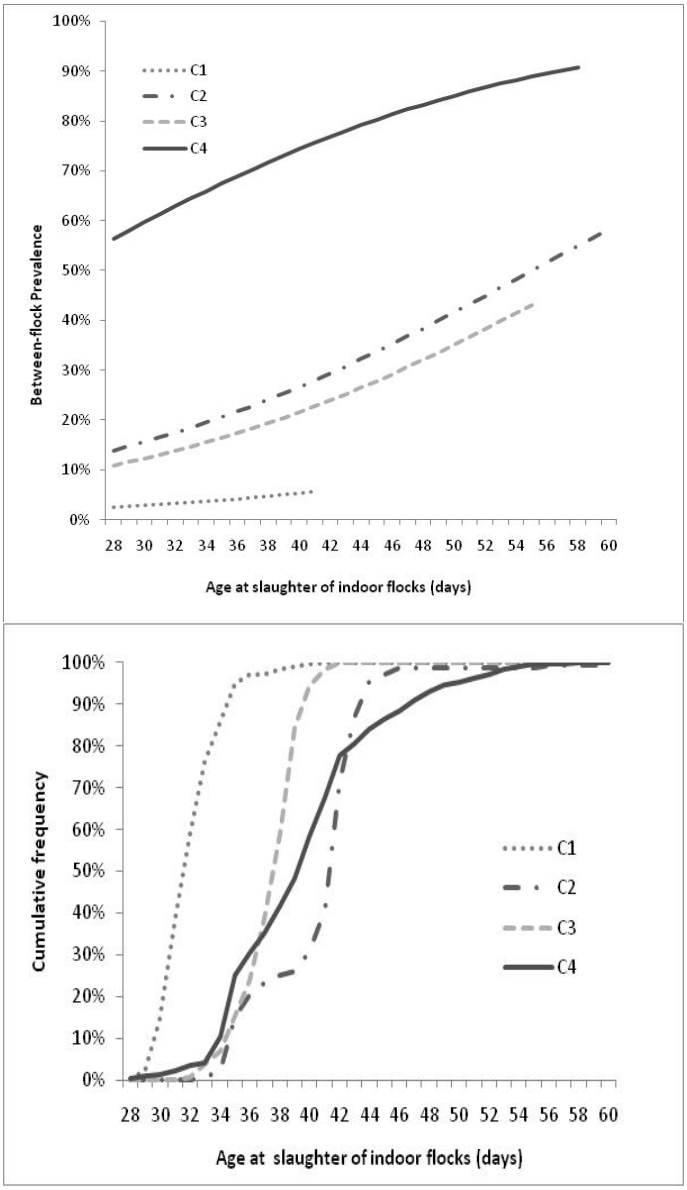


Figure 6: Effect of decreasing age at slaughter on between flock prevalence (BFP) of *Campylobacter*– upper panel; normalised frequency distribution of age at slaughter in 2008 – lower panel using EU baseline survey data (EFSA, 2010b). Maximum age at slaughter was restricted to 60 days to avoid extreme values. Public health risk reduction is proportionate to BFP reduction.

5.2.2.5. Reduced *Campylobacter* numbers in the intestines

Reduced *Camylobacter* numbers in the intestines could potentially be achieved by currently experimental interventions, including bacteriocins, bacteriophages, vaccination, or treatment of drinking water with organic acids, as discussed in Chapters 4.2.5 to 4.2.8. Assuming such interventions were available, the effect on human health of reductions of 1, 2, 3 and 6 $\log_{10}$ units in *Campylobacter* numbers in the intestines of both indoor and outdoor broiler flocks were evaluated. The public health benefits (assuming the same reduction on the carcasses) were over 65% for all countries with a reduction of 1 $\log_{10}$, above 91% if the reduction was 2 $\log_{10}$, above 98% with a 3 $\log_{10}$

reduction and 100% for 6 $\log_{10}$. However published data suggest that each $\log_{10}$ reduction in intestinal counts results in a lower reduction of 0.64 $\log_{10}$ on the meat. This would still yield at least public health risk reductions of 48%, 76%, 90% and 100% in human cases respectively. This is obtained using the classic + DR model, for the other two DR models, public health benefit is lower.

Table 7: Effect of 1, 2, 3 and 6 $\log_{10}$ reduction of *Campylobacter* in caecal contents of broilers, both for indoor and outdoor flocks, using the classic + DR model on the relative reduction in human cases

Red. caecal counts		**1 $\log_{10}$**	**2 $\log_{10}$**	**3 $\log_{10}$**	**6 $\log_{10}$**
Red. carcass		**1**	**2**	**3**	**6**
Country	C1	83.2%	97.5%	99.7%	100.0%
	C2	66.6%	91.9%	98.6%	100.0%
	C3	67.0%	91.7%	98.4%	100.0%
	C4	65.5%	91.0%	98.3%	100.0%

5.2.2.6. Decontamination in the slaughter-house

Several studies were available with good data on reduction of *Campylobacter* counts on carcasses as a consequence of applying decontamination by chemical or physical means. The results are summarized in Table 8. In general, there is an increase in the risk reduction obtained with increasing reduction of carcass counts. Results for the four countries are very similar. The results are sensitive to the dose-response model applied. The classic+ DR model generally predicts a greater risk reduction than the "simple exponential" and "modified Beta Poisson" DR models in CAMO.

A reduction of approximately 0.5 $\log_{10}$, as documented for treatment with lactic acid would result in a risk reduction of between 37 and 56%. Note that, as discussed in Section 4.6.4.1, the effect of lactic acid could increase if it was not immediately washed off the carcasses after treatment. A 1 $\log_{10}$ reduction (e.g. by short-time freezing) was predicted to result in a 65-83% risk reduction, whereas hot water treatment (1.25 $\log_{10}$ reduction) would result in a 75-89% risk reduction. Clearly, irradiation is the most effective decontamination treatment, reducing the risk by virtually 100%, whereas long term freezing is the next most effective option (87-98% risk reduction). The relative risk reductions are very similar for C2, C3 and C4, but in general higher for C1 (see Figure 7).

Table 8: Effect of decontaminations in slaughter-house on the relative reduction in human cases in the four study countries

Treatment	**Effect ($\log_{10}$ reduction)**	**Reduction in public health risk**
Irradiation/cooking	6.00	100%
Short time freezing: Georgsson *et al.* (2006), Rosenquist *et al.* (2006), Sandberg *et al.* (2005),	0.91 - 1.44	62 – 93%*
Long time freezing: Sandberg *et al.* (2005), Georgsson *et al.* (2006)	1.77 - 2.18	87 – 98%
Lactic acid: Bolder (2007)	0.47	37 – 56%
Hot water: Corry *et al.* (2006)	1.25	75 – 89%
Acidified sodium chlorite: Bashor *et al.* (2004), Kemp (2001),	1.26 – 1.75	75 – 96%
Trisodium phosphate: Bashor *et al.* (2004)	1.03	67 – 84%

* Range of expected reduction in public health risk in four study countries

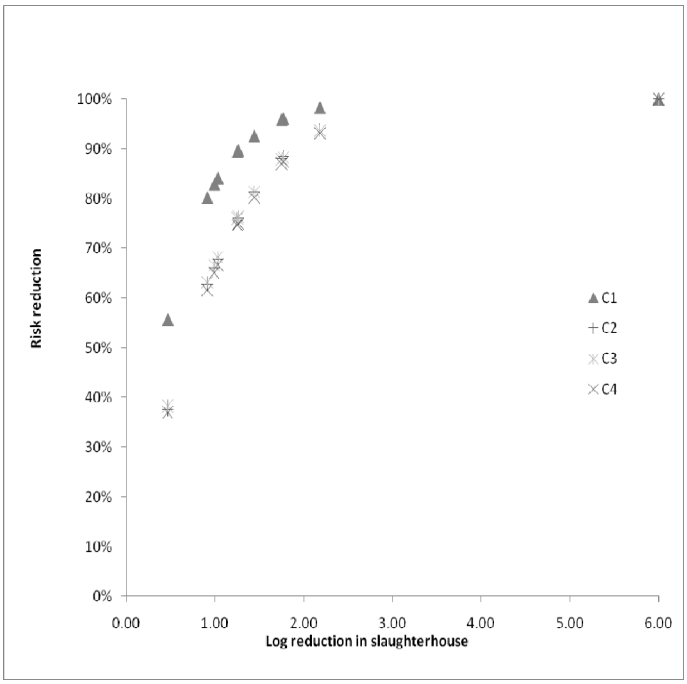


Figure 7: Risk reduction using decontamination treatments in the four study countries

5.2.2.7. Scheduled slaughter

The efficacy of scheduled slaughter is dependent on the ability of the *Campylobacter* detection method used to accurately determine flock positivity at the time of slaughter. The effect of scheduled slaughter was evaluated for two different potential detection protocols. Detection by culture needs three to four days in the laboratory and was assumed to involve sampling one week before slaughter. In this time period, flocks that are not (detectably) colonized at the moment of sampling, may still become colonized before slaughter. Detection by PCR needs only one day in the laboratory and was assumed to involve sampling four days before slaughter. In this shorter time period, the risk of a flock becoming colonized is smaller. Data on the sensitivity of the two protocols were available for Norway. Hofshagen and Kruse (2005) indicate that the culture-based protocol has a sensitivity of 48%, whereas Hofshagen and Opheim (2008) indicate that the PCR-based protocol has a sensitivity of 75%. Flocks in which *Campylobacter* was detected were assumed to be subjected to four different possible treatments: irradiation, freezing (short- and long-term) and lactic acid treatment.

If R is the risk associated with broiler meat before applying an intervention to all flocks and R' is the risk after applying the intervention, then the risk reduction due to the intervention can be defined as $\Delta R = R'/R$. Then, if the sensitivity of the detection protocol is Se, the risk reduction by scheduled slaughtering is $\Delta R_s = Se.\Delta R$. Risk reduction was clearly less than when applying the intervention to all batches when using culture-based detection. With the increased sensitivity of the PCR-based detection, the impact of scheduling on risk reduction is less limited. The benefits of scheduling increases with decreasing BFP, as the intervention is then applied to a small proportion of the flocks. The fraction of flocks to which treatment would be applied was 57% for the C4, 23% for C2 14% for C3 and 2% for C1.

Table 9: Risk reduction from scheduled slaughter in C1* using the classic + dose-response model

Intervention	Risk reduction with scheduling based on the effect of decontamination at slaughter		
	Treatment of all flocks	**Scheduling with PCR detection (sampling 4 days before slaughter)**	**Scheduling with culture detection (sampling 7 days before slaughter)**
Irradiation	100%	75%	48%
Long term freezing	98%	74%	7%
Short term freezing	83%	62%	40%
Lactic acid	56%	42%	27%

* Results for the other three countries are in the modelling report in Appendix C.

5.3. Validity and limitations of used quantitative models

The validity of the risk assessment models depends on the reliability of the model structure, its inputs, the underlying assumptions and the correct interpretation of the results. Two principles are considered: "Fit for purpose", i.e. the risk assessment product should be suitable for the intended purpose, and "Right first time", i.e. all possible errors are identified and eliminated. The analysis of the validity of the models used includes the quality of data used and how they are assembled, the relevance of the assumptions, the quality of the final assessment results and their interpretation, the implementation and the verification that all tasks incorporated in the different steps of the assessment model have been conducted in a technically correct manner.

The approach used in this risk assessment requires firstly that the distribution of *Campylobacter* concentration on carcasses after chilling and the between flock prevalence be linked with the public health risk; secondly that the effect of interventions on the distribution of *Campylobacter* concentration on carcasses after chilling and the between-flock prevalence should be quantified; and subsequently used to assess the expected risk reduction.

The expected changes are defined as the difference from a basal state ("current situation") defined as the concentration distributions and the between-flock prevalences assessed from the EU baseline survey data to assess. CAMO is designed to avoid explicit modelling of the consumer phase. A modified dose-response model is used to link the carcass concentration and the probability of campylobacteriosis. In other words, CAMO does not include formal consumer model nor a formal exposure assessment. CAMO was modified by the working group to include the missed components by using the classic + model developed by Nauta and Christensen (2011). The classic + DR model integrates consumer model, exposure assessment and dose response model.

5.3.1. Validity

The public health risk is the result of the integration of between-flock prevalence (BFP), concentration distribution parameters, consumer practices, cross contamination characteristics and dose response model. Two approaches are tested: i) empirical approach, and ii) mechanistic approach.

The CAMO structure predicts a direct link between the BFP and public health risk, hence the expected risk reduction is derived directly using the following formula:

$$Risk\ reduction = \frac{BFP_{current} - BFP_{new}}{BFP_{current}},$$

where BFP_{new} is the expected BFP after the implementation of the considered invervention scenarios. This simplification is possible because the model assumes 100% intra-flock prevalence, that BFP and level of carcass contamination are independent and no interaction between subsequnet flocks.

CAMO uses an empirical approach, in which the link between carcass contamination (prevalence and concentration, as measured in the EU basline study (EFSA, 2010a) is assessed empirically with the

observed number of human campylobacteriosis cases in the different MSs. Two dose response models were used, the "simple exponential" and "modified Beta-Poisson". The first model has one parameter, r, which was assessed for each MS. The second model has two parameters that are adopted from the literature on the relation between ingested dose and probability of infection (Black *et al.*, 1988). They are assumed to be constant and not specific to each MS. To take into account that not all the *Campylobacter* present in one carcass will expose the consumer an additional parameter, b, was added and assessed for each MS. The parameter (b) is included in the model in a way that leads to two possibilities: 1) the total amount of *Campylobacter* reaches the consumer 2) none of the *Campylobacter* will reach the consumer. The value of b has no impact on the relative risk estimates on the effects of interventions.

The classi + approach uses the same Beta-Poisson model parameters as in the CAMO model but includes a mechanistic consumer phase model (including transfer rate and cross-contamination) as described by Nauta *et al.* (2008) and Nauta and Christensen (2011).

For the two approaches the BFP is included as a multiplying factor.

As the the objective of this mandate is to characterize the change in, but not the absolute, public health risk, it is useful to conduct a sensitivity analysis assessing the impact of approach choice on the public health risk reduction. To simplify the sensitivity analysis, the consumer phase module is summarised as a binomial process with the number of trials equal to the number of *Campylobacter* present on one carcass. The probability for one cell to be ingested by one consumer is assumed variable between consumers and following a beta distribution. Different sets of beta parameters were used. For CAMO the beta distribution was replaced by the mean of the beta distributions used in the sensitivity analysis. What-ever is the parameter used of *Campylobacter* transfer, CAMO always gives the same risk reduction.

The programming of the model should achieve three goals: internal validity, usability, and extendibility. The first goal verifies if the program is correctly implemented. This verification was partly evaluated in the peer review process, but the complexity of the model including the use of not accessible macro functions did not allow a full verification. In the peer review, some unexpected results were observed and part of them were attributed to mistakes in the programming. The detected errors were fixed by the contractor.
The second goal is about the usability and the possibilities given to members of the ad-hoc Working Group and the BIOHAZ Panel to understand how the model is functioning. The interface provided by the contractors and the traceability for different modifications from old versions to new versions is well described. However, the explanations given in the final report are not sufficient and the user needs to know Excel functions and function syntaxes in the proprietary software to understand the different steps of the model in detail.
The third goal is about the extendibility which allows the possibility to adapt the program for new uses or modification. As it is implemented, the model is able to include different new parameters and modification of the dose response model.

5.3.2. Limitations

The Dutch econometrist Henri Theil once remarked that "models are to be used, but not to be believed". When interpreting the results of any risk assessment model, including the models presented in this Opinion, it is important to realise their limitations.

Even though the models are based on EU-wide data for the occurrence of *Campylobacter* in the broiler meat chain as well as on human illness, many simplifying assumptions were made to construct the risk assessment models. No generic model exists that is applicable across the EU. Humans become infected by various mechanisms including improper cooking and cross contamination - these are not explicitly included in CAMO. On the other hand, applying the classic+ DR-model assumes that consumer practices are similar in all MSs and remain stable. This assumption may not be realistic, and could be relaxed if data become available. The host status is not considered, e.g. pre-existing acquired

immunity, greater susceptibility in children and the elderly are not ackowledged. The data available for dose-response estimation derive from a study (Black *et al.*) on adults. As a consequence, the absolute risk estimates are highly uncertain, and are not presented as such in this Opinion. The estimates of public health risk reduction are more robust, as many uncertain factors occur both in the numerator and the denominator and uncertainties are cancelled out to some degree. Some of the key limitations in the current risk assessments are discussed below.

5.3.2.1. Longitudinal correlation of quantitative data

The data from the EU baseline survey are available only after chilling. The contamination at previous steps in the production chain was estimated by subtracting the effect of the different operations from the contamination level observed in the EU baseline survey. Once the initial contamination load, just before the application of the management option being considered, is estimated, it is possible to estimate what would be the final contamination level under different scenarios of intervention. Moreover, because the back calculation of level of contamination is conditional on the current application of management options, their potential effect is estimated using the same principle. As the calculations are done at MS level or EU level, the effect of the different slaughtering operations and the different interventions are subtracted directly from the overall distribution of the concentration, not taking into account the possible local (slaughter-house) situation and practices. The variability of the effect of interventions between slaughter-houses is also ignored. The predicted changes in the parameters (moments) of the probability distribution of contamination level after chilling associated with the application of control options need to be validated at MS level and slaughter-house level using longitudinal data whenever possible.

Quantitative longitudinal data that have proven to be particularly helpful for the development of risk assessments include, for example Oosterom *et al.* (1983), Izat *et al.* (1988), Mead *et al.* (1995), and Berrang and Dickens (2000), which provide the changes in mean (and standard deviation) of concentrations for a set of flocks.

Quantitative longitudinal data for *Campylobacter* during processing is now increasingly available (Guerin *et al.*, 2010). However, comparative studies and meta-analysis of such longitudinal data are lacking. In general, comparison of such studies is complicated by the use of different methods for microbiological and statistical analysis, such as different sample types and sampling points (caecal samples, transport container swabs, pericloacal swabs, carcass washes, carcass swabs, neck skins, etc.) and pooling. It is not easy to combine the presence / absence data and quantitative data from flocks along the food chain. The development of appropriate statistical analysis and standardization to allow a combination of such data is required. This lack of coherent data along with the variability in farm management, slaughtering practices and hygiene implementation between countries and broiler industry complicates the validation of risk assessment at each stage of the processing model.

In general, it is expected that high prevalence and concentrations of *Campylobacter* at primary production and slaughter will result in relatively high prevalence and concentrations on the meat products. This is particularly the case for *Campylobacter*, because it is assumed to multiply only in the intestinal tract of living birds. A reasonable hypothesis is, therefore, to expect that prevalence and concentrations found in different flocks correlate well along the broiler meat production chain.

Studies for correlations between concentrations in the broiler meat production chain show contrasting results. Several authors find a significant positive correlation: Fluckey *et al.* (2003) found strong correlations between *Campylobacter* status in live animals by drag swab sampling on the farm the day before slaughter and in caecal samples in the processing plants. Caecal contents therefore proved to be a good indicator of the *Campylobacter* status in broiler farms. Rosenquist *et al.* (2006) found a positive correlation between *Campylobacter* concentration in intestinal content and carcasses (neck skin) after defeathering. Reich *et al.* (2008) observed a positive correlation between the number of *Campylobacter* present in the caeca and the number of bacteria present on carcasses and cut products. However, others do not find these correlations. Allen et al (2007a) found no overall correlation between the numbers of *Campylobacter*s detected in caeca and on carcasses and Hansson et al (2007b)

found no correlation between neck skin samples and carcass wash data. Nauta et al (2009b) found that the *Campylobacter* concentration on breast fillets did not correlate with that of caecal contents, but for crate samples and breast fillets, and container samples and caecal contents a (rather weak) significant correlation was found.

Additional longitudinal studies have been published, e.g. by Rosenquist et al (2006), Berrang et al (2007), Allen et al (2007a), Klein *et al.*, (2007) and Nauta et al (2009a) which showed that there seems to be a general trend in that processes such as scalding, washing and chilling lead to a reduction in *Campylobacter* counts on carcasses whereas operations like defeathering and evisceration cause an increase in *Campylobacter* counts due to leakage of intestinal contents during these processes. Despite changes in *Campylobacter* counts over consecutive process operations, a general decline of the *Campylobacter* concentrations towards the end of the processing line is found in most studies. The net result will, however, depend on the process equipment, and how this fits to the size of the birds. Also the hygienic measures applied in a given slaughter-house are likely to have a major impact on the final numbers of *Campylobacter* on the carcasses. The variations in changes in *Campylobacter* counts have to be accounted for in risk assessment.

Hence, it appears that different studies yield different results and although positive correlations between faecal or caecal samples, skin samples and meat products are frequently found, this correlation is not obvious, certainly when meat product samples are involved. So far it is not clear what causes the differences between studies. They may be attributable to differences in processing practices between countries, but could also be caused by different study designs, different methods for statistical analysis and differences in prevalences and concentrations (and the variation therein) of *Campylobacter* in different regions. Other sources of differences in results may be randomness, seasonality, differences between strains, differences between sampling protocols, differences between sampling types, etc.

5.3.2.2. Test characteristics

Investigation of the detection and enumeration test results used to estimate the prevalence of *Campylobacter*-contaminated broiler carcasses showed that the diagnostic sensitivities of both tests varied significantly between Member States. All tests have imperfections, and even within the confines of a harmonised survey protocol there exists potential for imperfections to be accentuated in some circumstances, e.g. a specific laboratory environment. Test imperfections and uncertainty in sensitivity and specificity estimates should not be overlooked when interpreting a test-based prevalence estimate. This diagnostic dilemma can be further explored from a recent study by Edson *et al.* (2009), who presented results from proficiency testing of *Campylobacter* spp. detection in U.S. food laboratories (which ranged from 380 to 442) over the period from 1999 to 2007. The cumulative 9 years "false-negative" rate was 13.6% (and specifically for *C. coli* it was 24.0%). Such a rate of false-negatives is considerably higher than the rate of 5.9% for *Salmonella* spp. and 7.2% for *Listeria monocytogenes*, as reported in the same study.

One possible additional confounder for test sensitivity is the presence of *Campylobacter* bacteriophages (Connerton *et al.*, 2004). Such viruses are present in broiler samples and could trigger the lysis of *Campylobacter* in a type-specific manner once the bacteria were stimulated into growth. This factor might influence the recovery of some strains from both caeca and carcasses.

In light of the above, it is notable that in many *Campylobacter* QMRA models the risk assessors have taken the apparent prevalence (test-based prevalence) data to be equivalent to the true prevalence data. This might be of serious impact when considering intervention scenarios aimed at reducing the prevalence in a food chain module (e.g. reducing flock prevalence or prevalence at carcass level), as decisions can be biased by diagnostic test imperfections. Low (detection method) test sensitivity implies that the true prevalence of *Campylobacter*-contaminated carcasses might be underestimated for some MSs. Thus, it might be valuable in these MSs to consult statistical and modelling-based approaches (e.g. Bayesian statistical modelling) in order to have a better estimate of the apparent prevalence measured in this survey. An example of such an approach is that used in the case of

Campylobacter in the broiler sector in the Netherlands and Belgium (Habib *et al.*, 2008; Woldemariam *et al.*, 2008). However, attempts to apply such methods to the EU baseline survey data have not been succesful.

5.3.2.3. Strain variability

To date all *Campylobacter* risk assessments have assumed that all *Campylobacter* strains and variants have identical properties. However, strain variation may be important as different strains might have different virulence and physiologic properties (e.g. survival, pathogenic characteristics).

There is ample evidence to demonstrate diversity amoung *Campylobacter* strains at both the genomic and phenotypic levels. Whether this diversity is reflected in variable characteristics which would affect the outcome of risk assessments has yet to be determined. There is some epidemiological evidence that certain strains (e.g. some *C. coli* strains) can survive better in the environment, especially in surface waters (Kemp *et al.*, 2005). Other species, e.g. *C. lari*, preferentially colonize avian intestines. Genetic typing techniques, like multilocus sequence typing (MLST), is also beginning to show host adaptation for *C. jejuni* strains. However, such associations are yet to be fully explored.

Molecular typing has been used to investigate the diversity of *Campylobacter* strains in live chickens and on matching carcasses. As with all such studies the sensitivity is limited by the number of isolates tested and it can be assumed that only the predominant strains present will be detected. However, such studies indicate that the diversity of strains varies considerably along the food chain. Broilers may become colonized with both *C. jejuni* and *C. coli*. At about six weeks the majority of strains isolated from broilers is *C. jejuni* but in older animals, e.g. during organic production, there is a shift towards *C. coli* (El-Shibiny *et al.*, 2005). In some studies most of the flocks are infected with multiple strains but this seems to be country and management system related. In UK studies usually one to three strains were isolated (Ayling *et al.*, 1996; Shreeve *et al.*, 2000), while in a Dutch study (Jacobs-Reitsma *et al.*, 1995) a maximum of 4-5 strains was observed. Clearly, multiple strains can colonize a single flock at the same time and longitudinal studies (Shreeve *et al.*, 2000) suggest that these sequentially colonize the flock presumably as a consequence of multiple exposures from horizontal sources. Experimental studies suggest that such shifts are a reflection of homologous competitive exclusion (Chen and Stern, 2001), and that some strains have a greater capacity for survival and growth in the chicken gut than others. Additional explanation might be the effect of bacteriophages on the *Campylobacter* population in vivo (Connerton and Connerton, 2006) and acquired immunity (Cawthraw *et al.*, 1994). Because of a greater exposure to the environment, free-range flocks tend to be colonized with an even greater diversity of strains during rearing. The extent of diversity in any flock will therefore be dependent on the age of the flock and the exposure to potential sources. Nevertheless, some strains appear to predominate in a flock (Thomas *et al.*, 1997). Such mixed strain infections may lead to even more variety in *Campylobacter* strains as they may exchange DNA leading to chimera strains thus increasing strain diversity (de Boer *et al.*, 2001). Some strain diversity also occurs due to genetic instability during colonization. The study of Ridley *et al.* (2008b) suggests that during *Campylobacter* growth in the chicken gut genomic rearrangements occur, enabling strains with varying capacities for colonization to be generated.

During transport, birds are exposed to various strains in the crates and the diversity of strains on the bird surface increases. Similarly during processing the carcasses are exposed to cross-contamination throughout the abattoir. Interestingly, not only are strains gained but the strain(s) colonizing the flock may also be considerably reduced, presumably due to poor survival properties (Newell *et al.*, 2001). Thus, the strains isolated from carcasses may not reflect the strains originally in the flock at the farm or even at slaughter.

The dynamics of strain diversity in flocks and on retail carcasses is therefore complex and fluid. As a result it is unlikely that the total gene pool of chicken *Campylobacter* has yet been sampled for risk assessment and epidemiological attribution purposes. Strain diversity may reflect variation in phenotypic properties such as infectivity, virulence and stress responses. Such properties could determine survival times in the farm environment (thus affecting flock colonization potential),

antibiotic resistance (which might become important in environments contaminated by antibiotics), persistence on carcasses or susceptibility to decontamination methods or resistance to antibodies or bacteriophages (thus affecting the number of organisms with disease causing potential). Currently, no data is available to quantify these strain differences and there is little, if any, evidence that strains with specific geno- or phenotypes have a greater disease-causing potential than others. Current intervention strategies target all *Campylobacter* strains found in poultry. As far as can be determined at present, such strategies should effectively reduce the overall public health risk without inducing specific risks.

6. "Standards" for *Campylobacter* in the broiler meat chain

6.1. Criteria, targets, objectives and current EU regulations

In a previous Opinion, the BIOHAZ Panel discussed the relationship between microbiological criteria, as currently applied in EU food safety legislation, and newer metrics proposed by the Codex Alimentarius Commission (EFSA, 2007). Based on the 'formal risk analysis approach', concepts that have evolved include Appropriate Level of Protection (ALOP), Food Safety Objective (FSO) and Performance Objective (PO). Furthermore, this new framework emphasizes that Performance Criteria (PC), Process Criteria (PrC) and Microbiological Criteria (MC) should be scientifically based.

The ALOP represents the current public health status in relation to food safety. The original purpose of the FSO and PO was to translate the ALOP into levels of hazards in the food chain that could be communicated to and managed by the food industry. The BIOHAZ Opinion also discussed public health goals that are intended to inspire actions to improve the future public health status and reduce disease burden. In the present opinion, the specific public health goal may be derived from the second Term of Reference where it is expressed as "to obtain e.g. 50% and 90% reductions of the prevalence of human campylobacteriosis in the EU caused by broiler meat consumption or cross-contamination." As discussed in Chapter 1.1, this is understood as a reduction of true (estimated) annual incidence of human campylobacteriosis associated with the preparation and handling of broiler meat

At present, food safety standards in the EU include microbiological criteria (MC) and targets in primary production. Targets can be considered equivalent to Performance Objectives and are aimed at reducing the prevalence of *Campylobacter* in the broiler flocks, thus mitigating the risk for human campylobacteriosis at its main reservoir. Such targets could be set in a similar manner to the targets for *Salmonella* in Regulation (EC) No 2160/2003[15] on the control of *Salmonella* and other specified foodborne zoonotic agents. The Member States are obliged to establish national control programmes to meet the targets set and these control programmes have to be approved by the Commission.

Regulation (EC) No 2073/2005[16] on microbiological criteria for foodstuffs introduces two different types of criteria: Food Safety Criteria and Process Hygiene Criteria. Food safety criteria are set for products placed on the market and define the acceptability of an individual batch of food products. If the criteria are not met the product/batch has to be withdrawn from the market. Process hygiene criteria give guidance on, and are an indicator of, the acceptable functioning of HACCP-based manufacturing, handling and distribution processes. In fact they communicate the expected final outcome of the processes by setting end-of-manufacturing product criteria. They are incentives to create awareness and stimulate improvement in processing practices and hygiene in the production process. It sets indicative contamination values above which corrective actions are required in order to maintain the hygiene of the process in compliance with this regulation.

Microbiological criteria are useful for validation and verification of HACCP-based processes and procedures, and other hygiene control measures. In addition microbiological criteria are used to assess the acceptability of a batch of food, including the circumstances where there is insufficient knowledge of production conditions, e.g. at port of entry. In EU legislation, they are also used as a method of communicating the level of hazard control that should be achieved. Meeting microbiological criteria

[15] OJ L 325, 12.12.2003, p. 1–15
[16] OJ L 338, 22.12.2005, p. 60–82

offers some assurance that particular pathogens are not present at unacceptably high concentrations. Microbiological testing alone may convey a false sense of security due to the statistical limitation of sampling plans, particularly in cases where the hazard presents an unacceptable risk at low concentrations and/or low and variable prevalence. However, for *Campylobacter* in raw poultry meat, within-batch prevalence in contaminated batches is typically close to 100% and in this case, microbiological criteria may be efficient tools to reduce risk. Food safety is a result of several factors. Microbiological criteria should not be considered without other aspects of EU food legislation, in particular HACCP principles, and official controls to audit food business operators' compliance. Nevertheless, the level of contamination with *Campylobacter* of products (e.g. carcasses) produced from colonized broiler chickens is high, and can readily be quantified by simple microbiological plate counts. Furthermore, most if not all individual food items in such a batch will be contaminated, and consequently the statistical limitations of investigating a limited number of samples from a batch may be less influential in this case. The within-batch variability of bacterial counts is an important parameter defining the performance of sampling plans for *Campylobacter* on broiler meat.

In the present Opinion, several examples of setting targets and criteria will be explored and related to the *Campylobacter* risk reduction obtained for the individual MS taking into account the present situation of *Campylobacter* prevalence and levels as derived from the 2008 EU baseline survey for *Campylobacter* in broiler carcasses. Whether the criterion is to be a process criterion or food safety criterion is a risk management decision and will affect in particular the severity and the consequences in case of non-compliance to the criterion, and the time scale during which the risk reduction is expected to occur.

6.2. Targets in primary production

As discussed in Chapter 4, the public health benefits of controlling *Campylobacter* in primary broiler production are expected to be greater than control later in the chain as the bacteria may also spread from farms to humans by other pathways than broiler meat. There is, however, very little information about these pathways and quantifying the impact of interventions at farm level can only be done for broiler meat related cases. In general, the human health risk reduction is proportional in the reduction on between-flock prevalence (BFP) at the farm, as there is only negligible interaction between different broiler batches. However, it is assumed that measures aimed at reducing BFP are only applicable to indoor flocks. Hence, the overall risk reduction will be less than the reduction of BFP in indoor flocks, depending on the percentage of flocks with outdoor access. As the current flock prevalence as well as the proportion of flocks with outdoor access varies between MSs, the public health impact of an EU wide target for flock prevalence will also vary between MSs. Table 10 shows the expected risk reduction if BFP reached a target of 50, 25, 10, 5, 1%, or 0% (see Appendix C, 3.2. CAMPrev. The results demonstrate that the risk reduction is higher if the baseline level of flock prevalence is higher but that a high proportion of outdoor flocks probably limits the public health benefits of targets in primary production.

Table 10: Risk reduction if different targets for prevalence of *Campylobacter* in indoor broiler flocks are achieved in the EU.

Country		Current BFP[a]	Risk reduction if BFP would be reduced to					
			50%	25%	10%	5%	1%	0%
Austria	AT	47.8%	0.0%	47.7%	79.1%	89.5%	97.9%	100.0%
Belgium	BE	30.3%	0.0%	17.4%	67.0%	83.5%	96.7%	100.0%
Bulgaria	BG	33.1%	0.0%	24.5%	69.8%	84.9%	97.0%	100.0%
Cyprus	CY	31.7%	0.0%	21.2%	68.5%	84.2%	96.8%	100.0%
Czech Republic	CZ	61.1%	18.2%	59.1%	83.6%	91.8%	98.4%	100.0%
Denmark	DK	19.2%	0.0%	0.0%	47.9%	73.9%	94.8%	100.0%
Estonia	EE	2.0%	0.0%	0.0%	0.0%	0.0%	49.0%	100.0%
Finland	FI	4.1%	0.0%	0.0%	0.0%	0.0%	75.8%	100.0%
France	FR	75.1%	33.4%	66.7%	86.7%	93.3%	98.7%	100.0%
Germany	DE	48.6%	0.0%	48.6%	79.4%	89.7%	97.9%	100.0%
Hungary	HU	50.5%	0.9%	50.5%	80.2%	90.1%	98.0%	100.0%
Ireland	IE	80.7%	38.1%	69.0%	87.6%	93.8%	98.8%	100.0%
Italy	IT	63.9%	21.7%	60.9%	84.3%	92.2%	98.4%	100.0%
Latvia	LV	41.0%	0.0%	39.0%	75.6%	87.8%	97.6%	100.0%
Lithuania	LT	42.0%	0.0%	40.4%	76.2%	88.1%	97.6%	100.0%
Malta	MT	97.0%	48.5%	74.2%	89.7%	94.8%	99.0%	100.0%
Poland	PL	79.2%	36.9%	68.4%	87.4%	93.7%	98.7%	100.0%
Portugal	PT	82.9%	39.7%	69.8%	87.9%	94.0%	98.8%	100.0%
Romania	RO	76.5%	34.6%	67.3%	86.9%	93.5%	98.7%	100.0%
Slovakia	SK	70.6%	29.2%	64.6%	85.8%	92.9%	98.6%	100.0%
Slovenia	SI	77.7%	35.7%	67.8%	87.1%	93.6%	98.7%	100.0%
Spain	ES	87.7%	43.0%	71.5%	88.6%	94.3%	98.9%	100.0%
Sweden	SE	12.4%	0.0%	0.0%	19.6%	59.8%	92.0%	100.0%
The Netherlands	NL	24.2%	0.0%	0.0%	58.8%	79.4%	95.9%	100.0%
United Kingdom	UK	75.8%	34.0%	67.0%	86.8%	93.4%	98.7%	100.0%
Weighted EU average			**29.3%**	**61.6%**	**84.4%**	**92.1%**	**98.4%**	**100.0%**
Norway	NO	3.3%	0.0%	0.0%	0.0%	0.0%	69.5%	100.0%
Switzerland	CH	59.5%	15.9%	58.0%	83.2%	91.6%	98.3%	100.0%

[a]BFP: between-flock prevalence based on EU baseline survey indoor flocks (EFSA, 2010a)

6.3. Case studies on microbiological criteria

To evaluate the public health impact of setting a microbiological criterion (MC) for *Campylobacter*, we do not state whether the criterion will be a FSC or a PHC. We assume that the MC is applicable to every individual batch of food product placed on the market. Here, a batch is defined as in the EU baseline survey as "a group of broilers which have been raised in the same flock and which are delivered and slaughtered on one single day". Hence, one broiler house may produce more than one batch of meat, if thinning is applied or if depopulation is carried out over several consecutive days but a batch is never composed of meat from broilers from more than one flock. The maximum public health impact would be achieved if all batches were tested and those that do not meet the MC diverted

away from the fresh meat chain and received a heat treatment or similar treatment that destroys (almost) all *Campylobacter*. This scenario would result in the maximum risk reduction, or the Minimum Relative Residual Risk (MRRR). The value of any proposed microbiological criterion relies on how well it can be implemented. While the setting of any microbiological criteria is mainly driven by its ability to improve food safety, its effectiveness will be determined by its acceptance and by the food industry's willingness to implement it; for which factors such as economic feasibility, technical and organizational practicality play an important role. Corrective measures to be taken upon non-compliance of the microbial criterion may be debated. The costs of rejection and divertion of positive batches away from the fresh meat chain would be high and, particularly in summer, the majority of batches of broiler meat could be rejected. Risk managers may decide to subject positive batches to some kind of chemical or physical decontamination treatment (e.g. freezing) to reduce the levels of *Campylobacter* and thus the associated risk of campylobacteriosis, or – in the case of a PHC – to require improvements in processing hygiene from producers to reduce the prevalence and numbers of *Campylobacter* on broiler carcasses and derived poultry meat. Such decisions will imply less risk reduction, at least in the short term. In the longer term, the industry response may lead to increasing risk reduction but the exact timing and magnitude of the industry response cannot be predicted.

In the quantitative evaluation, it is assumed that samples are the same as those used in the EU baseline study, i.e. neck skins and breast skin combined. It is expected that, at least in part, sampling for control of *Campylobacter* will be combined with sampling for *Salmonella* so that a relatively large sample weight will be needed. For the EU baseline study 27 grams were taken from each carcass sampled, made up of the neckskin plus a strip of skin from the breast area of the carcass. It is important to use a standardized protocol for sampling as differences in levels of *Campylobacter* of up to 1 $\log_{10}$ unit may occur between the results of the analysis of neck skin versus the breast skin sampled, which may impact on the compliance or not to the microbial criterion. An alternative sampling method, which is better standardized, is to use carcass rinse instead of skin samples. Carcasses can be sampled in the abattoir by rinsing with sterile water, and then replaced on the line. Stern and Line (2009) got equivalent results for enumeration of *Campylobacter* and *Salmonella* when rinsing with Buffered Peptone Water, tap water or universal pre-enrichment broth. This would minimise the difference between carcasses with and without neck skin. There is an ISO method for carcass rinsing ISO 17604:2003/Amd 1:2009. In the absence of EU-wide data on carcass rinses, the data on skin samples will be used instead for the risk assessment. Note that this ignores any impact of sampling procedures on differences in counts between slaughter-houses or countries.

The risk assessment model (CAMC) used to evaluate the public health impact of an FSC is explained in detail in Appendix D. Briefly, the data from the EU baseline survey are assumed to represent the prevalence and level of contamination of all batches produced in one country. A Bayesian approach was used to fit a lognormal distribution to the observed counts, taking into account censored data, that is samples in which *Campylobacter* was determined to be present by enrichment but below the detection limit of the enumeration procedure. In the EU baseline survey, only one sample per batch was taken for enumeration, hence the observed variance is a mixture of within- and between-batch variance. Limited data are available on the relative size of these two variance components; in the default model the fraction of the total variance that is assigned to within-batch variance is set at 30%, and sensitivity analysis is applied to explore the impact of this assumption. Due to a lack of data, it is assumed that the within-batch variance is the same in all batchs in a country; the variation between batches in a country (due to e.g. season, differences between slaughter-houses) is described by variability in the mean concentrations in the flocks only. One-stage sampling plans are applied, with different values for m (the limit to microbiological counts in CFU/ gram of skin sample), n (the number of units comprising the sample) and c (the number of sample units on which the country may exceed m). Perfect test sensitivity and specificity are assumed. Two-stage sampling plans are not evaluated in this Opinion, as they would require a more complex modelling approach that would not be justified by the current data availability. It would be desirable to consider evaluating two-stage sampling plans in future. Further future improvements in this modelling approach might include the evaluation of other sampling strategies such as limiting the sampling to a fraction of all batches, either randomly or by applying a risk-based sampling strategy.

The percentage of batches not complying with the criterion (BNMC) is calculated to evaluate the public health impact of a MC. The BNMC depends on the (lognormal) distribution of mean concentrations in the batches, the within-batch variance, and the values of n, m and c applied for the MC. It can be calculated analytically by integrating the probabilities that the concentration found in a sample from a batch are larger than the m value for all possible concentrations in all batches, times the probability of finding such a batch, times the probability that it happens more than c times when n samples are taken. For each virtual batch, the BNMC will vary between MSs as well as for different values of m, n and c. Then, the public health risk associated with each batch is calculated using the Classic+ "dose-response"-model (see Chapter 5) and the MRRR is then calculated by comparing the average risk for all batches (i.e. current risk) with the risk associated with only those batches that meet the MC, in the theoretical scenario that all batches are tested and batches not complying with the MC would not be accepted for the fresh meat chain. A schematic representation of the modelling approach is provided in Appendix D.

Figure 8 shows the results of the evaluation of one potential MC (m= 1000, n=5, c=1) for all MSs, Norway and Switzerland. The current risk varies considerably between MSs, due to differences in the level of carcass contamination and batch prevalence. After application of a MC, the risk varies to a lesser extent between MSs. In MSs that had a baseline with low levels (at or below detection limit of the enumeration method) of *Campylobacter* per carcass and an associated low level of risk in the baseline situation, the relative risk reduction is small, though their risk continues to be low. The percentage of non-compliant batches is also low.

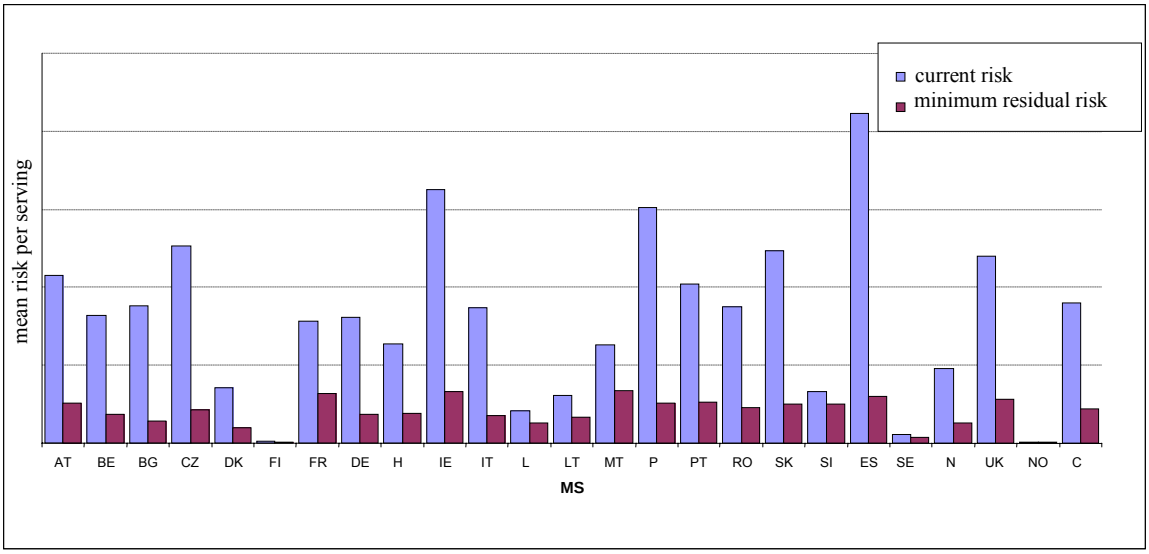


Figure 8: Comparison of the assessed mean risks per serving for the broiler meat produced in the different EU MSs as determined from the EU baseline survey data and the classic + DR model (current risks) and the minimum residual risks with implementation of the MC with m= 1000, n=5, c=1. Values of the mean risk are on a linear scale, but they are not given as they have to be read relative to each other. The MRRR value used elsewhere equals the value of the minimum residual risk divided by the current risk.

From Figure 9 it can be seen that risk reduction varies between 0 and 86% (MRRR = 14%), whereas batch rejection varies between 0 and 56%. Risk reduction and batch rejection are correlated to some extent. If the risk reduction and flock rejection are low, this usually means the current status in a MS is better (i.e. low counts in the EU baseline survey and/or a low batch prevalence). Note that for all MSs, the risk reduction is considerably larger than the proportion of batches that is rejected. This is related to the fact that more highly contaminated batches (with associated higher risk) have a larger probability of being rejected. At EU level, 26% of batches would not meet the MC and rejecting those batches would result in 71% risk reduction (MRRR = 29%).

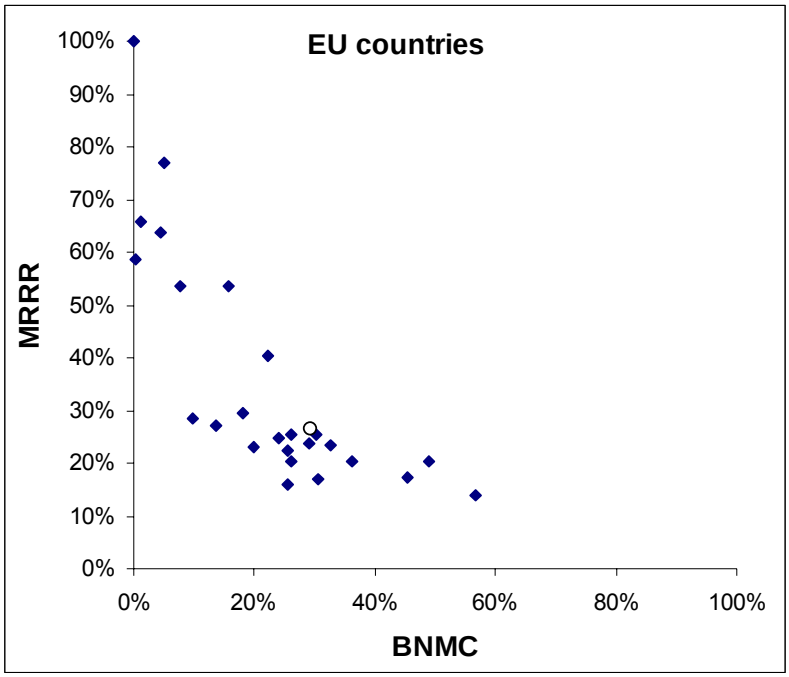


Figure 9: A scatter plot of percentage of batches not complying with the criterion (BNMC) and the minimum relative residual risk (MRRR) after implementation of the MC with m= 1000, n=5, c=1 for the different MSs. The EU mean is given by the small circle. Note that, ideally, both the MRRR and BNMC are low. The variability between the EU MSs is large. If the MRRR is high and the BNMC is low, this usually means the current status in a MS is better.

The impact of other sampling schemes for the MC was explored with m={100, 500, 1000, 5000, 10000} and {n,c} combinations {9{1,0},{3,0},{3,1},{5,0},{5,1},{5,2},{10,0},{10,1},{10,2}}. The EU mean was set as a default the results, see Figure 10.

First, it can be seen that the batch rejection decreases with an increasing limit of *Campylobacter* counts (m). This is an important output of the analysis, because the % positive batches are those where action should be taken, no matter how the MC is implemented (as FSC or PHC, for all batches or a (small) percentage of sampled batches only).

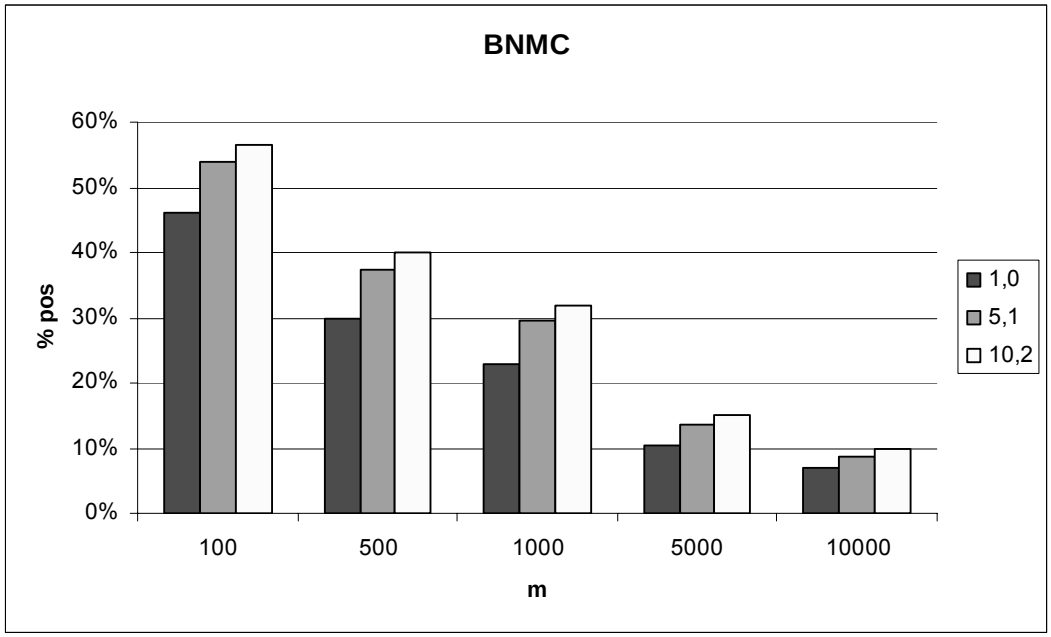


Figure 10: Batch rejection for different values of the threshold m, for three combinations of {n,c} = {1,0}, {5,1} and {10,2}. Results are the weighted EU means and will be different between MSs. BNMC – batches not complying with microbiological criteria.

The batch rejection and risk reduction are well correlated, as can be seen from Figure 11. This Figure shows results for the whole EU. The relationship differs greatly between countries, as is illustrated in Appendix D. Results in Appendix D. also show that an MC with n=3, c=0 performs slightly better than an MC with n=5, c=1, with both risk reduction and batch rejection slightly higher for the MC with n=3.
According to the model results, MCs that would result in at least 50% risk reduction for the EU as a whole are:

- any MC with m=100, 500, 1000;
- m=5,000 with n=3, c=0; n=5, c=0; or n=10, c=0; and n=10, c=1;
- m=10,000, n=10, c=0.

A risk reduction of at least 90% at EU level can only be obtained by an MC with

- m=100, n=3, c=0; n=5, c=0, 1 or 2 and n=10, c=0,1 or 2;
- m=500, n=10, c=0 and c=1.

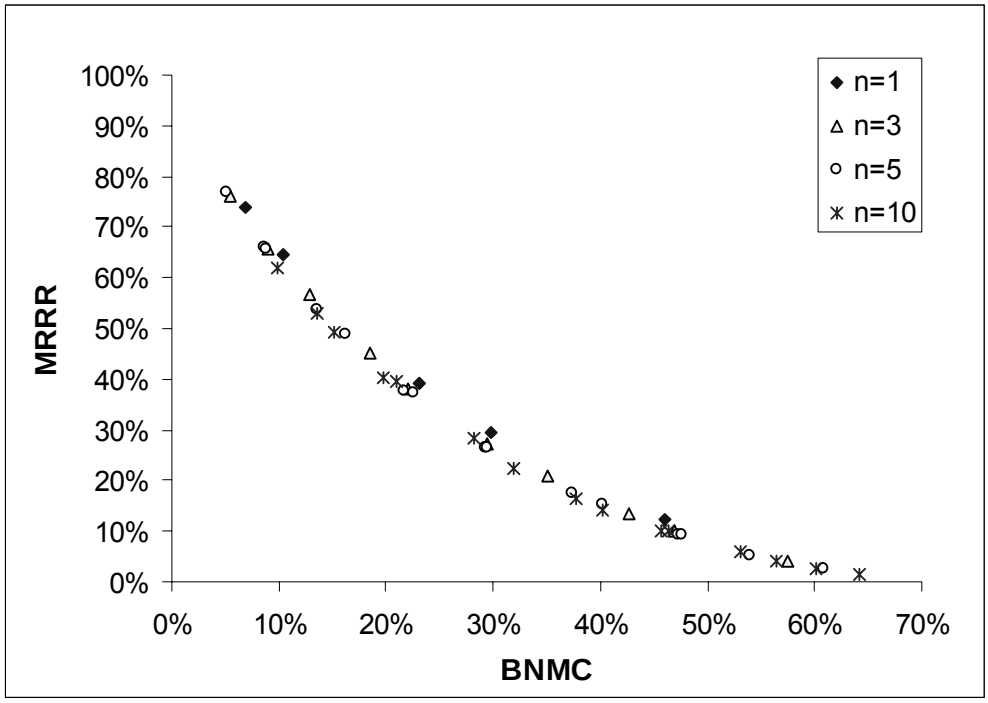


Figure 11: Batches not complying with microbiological criteria (BNMC) versus minimal relative residual risk (MRRR) for the weighted EU mean. When many batches do not meet the criterion, the residual risk once they are diverted away from fresh meat production is small; the residual risk is higher if fewer batches are diverted away. Increasing the sample size doesn't help very much. With higher m and higher c fewer batches are positive and the MRRR is higher.

Sensitivity analyses were done for several unknown parameters in the model. By varying the proportion of the total variance that was assigned to within--batch variance between 10 and 50%, the MRRR for the whole EU varied between 23 and 32%, and BNMC between 27 and 31%. A similar impact may be expected from the assumption that the within-batch variance was similar in all batches in all slaughter-houses in one MS. The risk reduction was sensitive to the ratio of carcass counts to counts on consumer products, which is unknown for most MSs. MRRR in the whole EU for m=1000, n=5 and c=1 ranged between 19 and 34% by varying this ratio 100-fold.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

It is estimated that there are approximately 9 (90% CI [3;20]) million cases of human campylobacteriosis per year in the EU27. The disease burden of campylobacteriosis and its sequelae is 0.35 [0.1;0.7] million disability-adjusted life years (DALYs) per year and total annual costs are 2.4 [0.9;5.1] billion €.

Campylobacter jejuni and *C. coli* are considered equivalent for the purpose of risk assessment in this opinion because there is no information on variability between these two species with respect to their behaviour in the food chain, impact of interventions or virulence for humans. There are no indications that *Campylobacter* strains with antimicrobial resistance behave differently in the food chain than their sensitive counterparts.

As previously estimated by EFSA, handling, preparation and consumption of broiler meat may account for 20% to 30% of human cases of campylobacteriosis, while 50% to 80% may be attributed to the chicken reservoir as a whole (broilers as well as laying hens). The transmission routes from chickens to humans, other than handling, preparation and consumption of broiler meat, are not well understood, and related public health benefits cannot currently be quantified.

The public health benefits of controlling *Campylobacter* in primary broiler production are expected to be greater than control later in the chain as the bacteria may also spread from farms to humans by other pathways than broiler meat. There is, however, very little information about these pathways and quantifying the impact of interventions at farm level was only done for broiler meat-related cases.

Answers to TOR 1: Identify and rank the possible control options within the broiler meat production chain (pre-harvest, at harvest and post-harvest), taking into account the expected efficiency in reducing human campylobacteriosis. Advantages and disadvantages of different options should be considered.

- Quantitative risk assessment based on data from four countries has concluded that there is a linear relationship between prevalence of *Campylobacter* in broiler flocks and public health risk.
    - Vertical transmission does not appear to be an important risk factor for colonization of broiler chickens with *Campylobacter*.
    - Biosecurity measures are considered essential to prevent flock colonization with *Campylobacter*.
    - The rigorous and continuous application of strict biosecurity measures that is necessary to prevent *Campylobacter* from entering the broiler house is mostly not achieved in the EU.
    - Colonization with *Campylobacter* of flocks with outdoor access is very likely to occur. Hence, the public health impact of biosecurity and related measures in primary production is restricted to flocks that are housed indoors. The risk estimates presented in this Opinion take the current mix of management systems in MSs into account.

- Where strict biosecurity measures are applied in indoor production, the use of fly screens effectively reduces flock colonization in summer and thereby reduces public health risk by 50 to 90% as estimated in one MS.
- Thinning (partial depopulation) is a risk factor for flock colonization. The public health impact of stopping this practice as estimated in four countries is expected to reduce the risk by up to 25%.
- Restricting the broiler slaughter age of indoor flocks to 35 or 28 days, as estimated from four countries, would reduce the public health risk by 10 to 20% or up to 50%, respectively.

- Quantitative risk assessment based on data from four countries has concluded that reducing the numbers of *Campylobacter* in the intestines at slaughter by 3 $\log_{10}$-units, would reduce the public health risk by at least 90%.
  - Inclusion of additives to feed or drinking water or vaccination, as preventive measures, could reduce flock prevalence as well as numbers of *Campylobacter* in the intestines. However, vaccination is still being developed, and there is conflicting evidence regarding the effectiveness of additives.
  - Based on experimental studies, administering bacteriocins or bacteriophages to broiler chickens 2-3 days prior to slaughter temporarily reduces the numbers of *Campylobacter* in the intestines of birds in colonized flocks by at least 3 $\log_{10}$-units.
  - These control methods are equally effective for birds with or without outdoor access.

- There are no data to quantitatively assess the effect of interventions related to transportation and holding before slaughter on the public health risk of *Campylobacter*. However, optimization of feed withdrawal, transportation procedures, and minimising holding time before slaughter, as stipulated by existing EU Regulations on animal welfare, will also reduce external bird contamination.

- Published risk assessments have shown that logistic slaughter, the separate slaughter, dressing and processing of negative and positive flocks, has negligible effect on human health risk.

- Quantitative risk assessment based on data from four countries has concluded that reducing the numbers of *Campylobacter* on the carcasses by 1 $\log_{10}$-unit, would reduce the public health risk by between 50 and 90%. Reducing counts by more that 2 $\log_{10}$ units would reduce the public health risk by more than 90%.
  - Although not quantitatively assessed, improvement of hygienic practices during slaughter is expected to result in a reduction in the level of carcass contamination.
  - Application of lactic acid, acidified sodium chlorite, or trisodium phosphate for carcass decontamination can significantly reduce numbers of *Campylobacter* on carcasses, compared to applying only water. It is estimated that the associated public health risk reduction is between 40 and 90%. Leaving these chemicals on the carcass might increase the effectiveness.
  - Hot water treatment of carcasses (80 °C for 20 sec) would result in a public health risk reduction between 50 and 90%.

- Long term freezing (2-3 weeks) of carcasses would reduce the public health risk by more than 90%, while short-term freezing (2-3 days) would result in a public health risk reduction of between 50 and 90%.
- Other decontamination techniques such as crust-freezing, steam or steam-ultrasound are being developed and there is currently insufficient data to assess their effectiveness.
- Irradiating using appropriate doses would eliminate public health risk.
- Cooking (parts of) carcasses on an industrial scale would eliminate public health risk if re-contamination is prevented.

- Scheduled slaughter aims to identify colonized flocks before slaughter so that they can be subjected to decontamination treatment. In low prevalence situations (winter in many MSs and also in summer in several MSs), the number of batches that need treatment is strongly reduced. Risk assessment, based on data from two countries, indicated that, when testing four days before slaughter, 75% of the colonized flocks are detected. The public health benefit will depend on the treatment applied to the positive flocks.

Strict implementation of biosecurity in primary production and of GMP/HACCP during slaughtering is expected to reduce the level of colonization of broilers with *Campylobacter*, and the contamination level of carcasses and meat from colonized flocks. The effects of such implementation cannot be quantified because they depend on many interrelated local factors. Nevertheless, their impact on public health risk reduction may be considerable.

Based on the results of the quantitative risk assessment, the specific control options discussed in this opinion can be ranked as follows in order of decreasing reductions in public health risk:

- 100% risk reduction by irradiation/cooking by reduction of carcass concentration by > 6 $\log_{10}$ units;
- More than 90% risk reduction by reduction of carcass concentrations by > 2 $\log_{10}$ units, which can be achieved by freezing for 2-3 weeks or reduction of the concentration in intestines at slaughter by > 3 log units;
- 50-90% risk reduction by reduction of carcass concentrations by 1-2 $\log_{10}$ units, which can be achieved by freezing for 2-3 days, hot water carcass decontamination or chemical carcass decontamination with lactic acid, acidified sodium chlorite or trisodium phosphate;
- 50-90% risk reduction by an equivalent reduction of flock prevalence, which has been achieved in one Member State by the use of fly screens in the presence of strict biosecurity measures;
- Up to 50% risk reduction by modifications of primary production, including restriction of slaughter age of indoor flocks to a maximum of 28 days; or by discontinued thinning.
- The risk reduction associated with reducing concentrations on carcasses is expected to be similar in all MSs (although the baseline level of risk differs considerably). The risk reduction associated with interventions in primary production is expected to vary considerably between MSs.
- Of these control options for reducing carcass concentration, freezing, hot water carcass decontamination and chemical decontamination are directly available from a technical point of view. It shoud be noted, however, that chemical decontamination is subject to approval in the EU and that currently no chemicals are approved for use.

- Of the control options in primary production, restriction of slaughter age and discontinuing thinning are directly available from a technical point of view. They do interfere strongly with current industrial practices, however.

Answer to ToR 2: Propose potential performance objectives and/or targets at different stages of the food chain in order to obtain e.g. 50% and 90% reductions of the prevalence of human cahempylobacteriosis in the EU caused by broiler meat consumption or cross-contamination. The performance objectives might include targets for reduction at pre-harvest and/or microbiological criteria for foodstuffs (qualitative or quantitative criteria for *Campylobacter* in general or for certain strains (e.g. species, resistant to certain antibiotics). In addition, guidance should be given on a realistic time period needed to achieve these reductions, taking into account the outcome of TOR1.

The answer to this TOR refers to the instruments that are currently available in EU legislation, i.e. targets at primary production and microbiological criteria for foodstuffs.

The realistic time period needed to obtain reductions due to targets in primary production will differ between countries depending on the present status and possibilities for practical implementation of different interventions and is a risk management issue.

It is not realistic to consider targets for flocks with outdoor access. The share of such flocks in the total production is expected to increase in future.

Microbiological criteria could theoretically be implemented immediately but the ability to comply will also differ between MSs.

- Quantitative risk assessment with data from all MS was used to evaluate the potential reduction in public health risk from *Campylobacter* related to setting targets for between-flock prevalence (BFP) or microbiological criteria.
    - Assuming all (5/25) countries with a BFP of less that 25% in 2008 maintain that status, then achieving a target of 25% BFP in all other MS is estimated to result in 50% reduction of public health risk at the EU level.
    - Assuming all (2/25) countries with a BFP of less that 5% in 2008 maintain that status, then achieving a target of 5% BFP in all MSs would result in approximately 90% reduction of public health risk at the EU level.
- Compliance with microbiological criteria is effective to reduce risks for *Campylobacter* on broiler meat because of high within-batch prevalences and low within-batch variability enabling detection of highly contaminated batches even when taking a limited number of samples. They stimulate improved control of *Campylobacter* during slaughter.
- The public health benefits of setting microbiological criteria were evaluated using data from the 2008 EU baseline survey. These estimates are average values for the whole EU; the impact could be very different between MSs.
    - Theoretically, a public health risk reduction > 50% at the EU level could be achieved if all batches that are sold as fresh meat would comply with microbiological criteria with a critical limit of 1000 CFU/gram of neck and breast skin. A total of 15% of all batches tested in the EU baseline survey of 2008 would not comply with such criteria.
    - Theoretically, a public health risk reduction > 90% at the EU level could be achieved if all batches that are sold as fresh meat would comply with microbiological criteria with a critical limit of 500 CFU/gram of neck and breast

skin. A total of 45% of all batches tested in the EU baseline survey of 2008 would not comply with such criteria.

RECOMMENDATIONS

- Effective control options should be selected and verified under conditions where the application is intended to be used by industry to reduce *Campylobacter* and comply with potential targets and/or MC when established.
- This opinion has identified several data gaps and therefore generation of data in several areas should be encouraged:
  - Biosecurity interventions in primary production under field conditions, including the effect of hygiene barriers and fly screens.
  - Improved slaughter-house hygiene and decontamination to assess their effect using naturally contaminated broiler carcasses under practical conditions.
  - Identify factors responsible for the variability of *Campylobacter* contamination among slaughter-houses in different MSs in order to identify new control options.
  - Potential improvement of efficacy of chemical decontamination (in particular lactic acid) by not washing off the carcasses after treatment.
  - Effect of combinations of interventions concurrently applied at different steps of the production chain.
  - Effective control options in outdoor production.
  - Data on within- and between-flock variance of the concentration of *Campylobacter* on carcasses after chilling, to allow more detailed evaluation of microbiological criteria.
  - Consumer behaviour, dose response, and the effects of acquired immunity to inform future risk assessments.
  - Public health impact of other poultry (e.g. laying hens, turkeys) as areservoir for human campylobacteriosis and transmission pathways between these species and humans.

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APPENDICES

A. UNDERREPORTING OF HUMAN CAMPYLOBACTERIOSIS IN THE EU

In 2008 there were approximately 191,000 notified cases of campylobacteriosis in the EU (EFSA, 2010c). Table 1 provides a summary of the reported data. The EU-average incidence rate of reported cases was 40.8 cases per 100,000 population.

Table 1: Reported human campylobacteriosis in EU-27, Norway and Switzerland in 2008 (EFSA, 2010c)

Country	Population	Reported human campylobacteriosis	
		Cases	Incidence rate
	(million)		(per 100.000)
Austria	8.319	4,301	51.7
Belgium	10.667	5,111	47.9
Bulgaria	7.640	19	0.2
Cyprus	0.789	23	2.9
Czech Republic	10.381	20,174	194.3
Denmark	5.475	3,470	63.4
Estonia	1.341	154	11.5
Finland	5.300	4,453	84.0
France	63.753	3,424	5.4
Germany	82.218	64,731	78.7
Greece	11.214	-	-
Hungary	10.045	5,563	55.4
Ireland	4.401	1,752	39.8
Italy	59.619	265	0.4
Latvia	2.271	0	0.0
Lithuania	3.366	762	22.6
Luxembourg	0.484	439	90.7
Malta	0.410	77	18.8
Poland	38.116	257	0.7
Portugal	10.618	-	-
Romania	21.529	2	0.0
Slovakia	5.401	3,143	58.2
Slovenia	2.026	898	44.3
Spain	45.283	5,160	11.4
Sweden	9.182	7,692	83.8
The Netherlands	16.486	3,341	20.3
United Kingdom	61.194	55,609	90.9
EU-27	497.528	190,820	40.8
Norway	4.737	2,875	60.7
Switzerland	7.593	7,877	103.7

Note: Population basis for calculating EU-27 IR excludes Greece, Portugal and is corrected for 52% coverage in the Netherlands

As discussed in a previous Opinion of the BIOHAZ Panel (EFSA, 2010d), there is considerable underascertainment and underreporting and the true incidence of human campylobacteriosis was estimated to range between 2 and 20 million cases per year. There is little information on specific underreporting factors in different MSs. In order to compare the risks associated with the prevalence and numbers of *Campylobacter* on broiler carcasses, as identified in the EU baseline survey (EFSA, 2010b) with human disease incidence, country-specific estimates of the true incidence of campylobacteriosis and the fraction attributable to consumption and handling of broiler meat would be required. For the purpose of this Opinion, the estimates on the true incidence were based on data describing differential risks to Swedish travellers as published originally by Ekdahl and Giesecke (2004). Updated information on the risk for Swedish travellers in the EU, as presented in Table 2, were obtained from the Swedish Institute for Communicable Disease Control (Smittskyddsinstitutet, SMI, Solna, Sweden). Cases reported in Table 2 were enumerated in the Swedish infectious disease surveillance system (SmiNet) and covered the years 2005-2009. Information on whether a case was travel-related or not, and the country of travel, was available for 91-97% of all reported cases with campylobacteriosis. Data on travel patterns of Swedish residents was obtained from the Swedish Travel and Tourism Data Base (TDB, Resurs AB, Stockholm, Sweden). More information on the survey methods and data processing is available at http://www.resursab.se/. Only journeys with an overnight stay in the country of destination were included in the model.

7,260 cases with campylobacteriosis were registered and approximately 46 million journeys were undertaken by Swedes. The average number of journeys per year was 9.2 million, higher than reported for 1997-2003 (7.5 million). Nevertheless, the number of reported cases of campylobacteriosis decreased from 1,786 to 1,452 per year. The average risk (per 100,000 travels) of campylobacteriosis in Swedish travellers returning from the EU in 2005-2009 was 15.9 (90% CI 15.6-16.2), ranging between 0.40 per 100,000 for travellers returning from Finland to 182 for travellers returning from Bulgaria. Uncertainty in the risk estimate was simulated by bootstrapping, assuming a Poisson process. Monte Carlo simulations were performed using @RISK 5.0 (Palisade Corporation, Ithaca, NY, USA), an add-in to Microsoft Excel®. Note that this approach takes only the sampling effects in case numbers into account. In the paper by Ekdahl and Giesecke (2004) uncertainty is estimated by a lognormal approximation, also taking sampling effects in the travel data into account. Such information was not currently available. However, bootstrapping of data as reported in Ekdahl and Giesecke showed only slightly lower uncertainty margins for the bootstrap approach. Apparently, uncertainty is dominated by sampling effects in the case numbers.

Table 2: Risks of campylobacteriosis in returning Swedish travellers, 2005-2009

Risk to Swedish travelers			
Country	*Cases*	*Journeys*	*Risk per 100,000 travels*
Austria	**66**	**924.831**	**7,14**
Belgium	**47**	**620.008**	**7,58**
Bulgaria	**931**	**511.366**	**182,06**
Cyprus	**115**	**592.149**	**19,42**
Czech Republic	**193**	**652.565**	**29,58**
Denmark	**233**	**6.884.980**	**3,38**
Estonia	**25**	**1.135.989**	**2,20**
Finland	**31**	**7.669.097**	**0,40**
France	**604**	**2.637.364**	**22,90**
Germany	**236**	**5.176.651**	**4,56**
Greece	**601**	**2.339.178**	**25,69**
Hungary	**231**	**501.369**	**46,07**
Ireland	**50**	**308.430**	**16,21**
Italy	**211**	**2.668.173**	**7,91**
Latvia	**29**	**577.638**	**5,02**
Lithuania	**15**	**113.304**	**13,24**
Luxembourg	**5**	**84.460**	**5,92**
Malta	**61**	**157.247**	**38,79**
Poland	**470**	**907.010**	**51,82**
Portugal	**265**	**585.442**	**45,26**
Romania	**99**	**90.340**	**109,59**
Slovakia	**17**	**51.691**	**32,89**
Slovenia	**8**	**92.058**	**8,69**
Spain	**2.430**	**5.889.338**	**41,26**
Sweden	**NA**	**NA**	**NA**
The Netherlands	**52**	**780.458**	**6,66**
United Kingdom	**235**	**3.702.100**	**6,35**
EU-27	**7.260**	**45.653.238**	**15,90**
Norway	**93**	**4.916.615**	**1,89**
Switzerland	**24**	**508.912**	**4,72**

NA: Not applicable

Estimates of the true incidence were anchored to population-based estimates from the Netherlands, based on raw data from a Dutch case-control study (de Wit *et al.*, 2001), where the incidence rates from these studies were applied to the average population of 2005-2009 and scaled to the observed average of laboratory-confirmed cases for these years in comparison to the year 1999 when the case-control study was performed. A full description of the simulation method is given in Havelaar *et al.* (2004), who used this approach for estimating STEC O157-associated gastro-enteritis in the Netherlands. An average of 81,300 (90% CI [29,400;173,000]) cases of campylobacteriosis per year were estimated to occur in the Netherlands between 2005 and 2009. The incidence rate in other MSs was calculated from this estimate as:

$$IR_C = (IR_{NL} \times ST_C) / ST_{NL}$$

where

IR_C = incidence rate of campylobacteriosis in country C (the Netherlands for C= NL)

ST_C = risk of campylobacteriosis for Swedish travellers to country C (the Netherlands for C= NL).

The true incidence of campylobacteriosis in a country was estimated by multiplying by the population size in 2009, as reported by EUROSTAT. An underreporting factor can then be estimated by comparison of the estimated true incidence with the reported incidence as represented in Table 1. The % of reported cases was then calculated as the inverse of the underreporting factor. The incidence rate of human campylobacteriosis in Sweden cannot be calculated from this data set, and was assumed to be the same as in Finland.

Table 3: Estimated true incidence of human campylobacteriosis in EU-27, Norway and Switzerland

Country	Swedish travellers		True incidence		Underreporting	
	Risk		Cases	Incidence rate	factor	% reported
	(per 100,000)	(relative to NL)		(per 100.000)		
Austria	7.14	1.071	43,964	528	10.2	9.8%
Belgium	7.58	1.138	59,880	561	11.7	8.5%
Bulgaria	182.06	27.325	1,030,036	13,482	54212.4	0.0%
Cyprus	19.42	2.915	11,347	1,438	493.4	0.2%
Czech Republic	29.58	4.439	227,360	2,190	11.3	8.9%
Denmark	3.38	0.508	13,721	251	4.0	25.3%
Estonia	2.20	0.330	2,185	163	14.2	7.0%
Finland	0.40	0.061	1,586	30	0.4	280.7%
France	22.90	3.437	1,081,207	1,696	315.8	0.3%
Germany	4.56	0.684	277,569	338	4.3	23.3%
Greece	25.69	3.856	213,360	1,903	-	-
Hungary	46.07	6.915	342,725	3,412	61.6	1.6%
Ireland	16.21	2.433	52,833	1,200	30.2	3.3%
Italy	7.91	1.187	349,136	586	1317.5	0.1%
Latvia	5.02	0.754	8,443	372	-	-
Lithuania	13.24	1.987	32,999	980	43.3	2.3%
Luxembourg	5.92	0.889	2,122	438	4.8	20.7%
Malta	38.79	5.822	11,778	2,873	153.0	0.7%
Poland	51.82	7.777	1,462,631	3,837	5691.2	0.0%
Portugal	45.26	6.794	355,915	3,352	-	-
Romania	109.59	16.448	1,747,108	8,115	873553.8	0.0%
Slovakia	32.89	4.936	131,537	2,435	41.9	2.4%
Slovenia	8.69	1.304	13,038	644	14.5	6.9%
Spain	41.26	6.193	1,383,619	3,055	268.1	0.4%
Sweden	NA	0.061	2,749	30	0.4	279.9%
The Netherlands	6.66	1.000	81,340	493	24.3	4.1%
United Kingdom	6.35	0.953	287,654	470	5.2	19.3%
EU-27	15.90	2.39	9,227,842	1,855	48.4	2.1%
Norway	1.89	0.284	6,636	140	2.3	43.3%
Switzerland	4.72	0.708	26,519	349	3.4	29.7%

For the EU-27, the incidence of campylobacteriosis is approximately 9.2 (90% CI [3;20]) million cases, which fits well in the range reported before. Also note that for the UK, the underreporting factor is estimated at 5.2 (90% CI [1.9-11]). An independent estimate of 7.6 in the mid-1990s, based on the Infectious Intestinal Disease study (Wheeler *et al.*, 1999) was similar. The underreporting factor for the EU as a whole is estimated at approximately 48 (90% CI [17-103]), but ranges between 0.4 for Finland and Sweden to almost 900,000 for Romania. The incidence data are visualised in Figure 1.

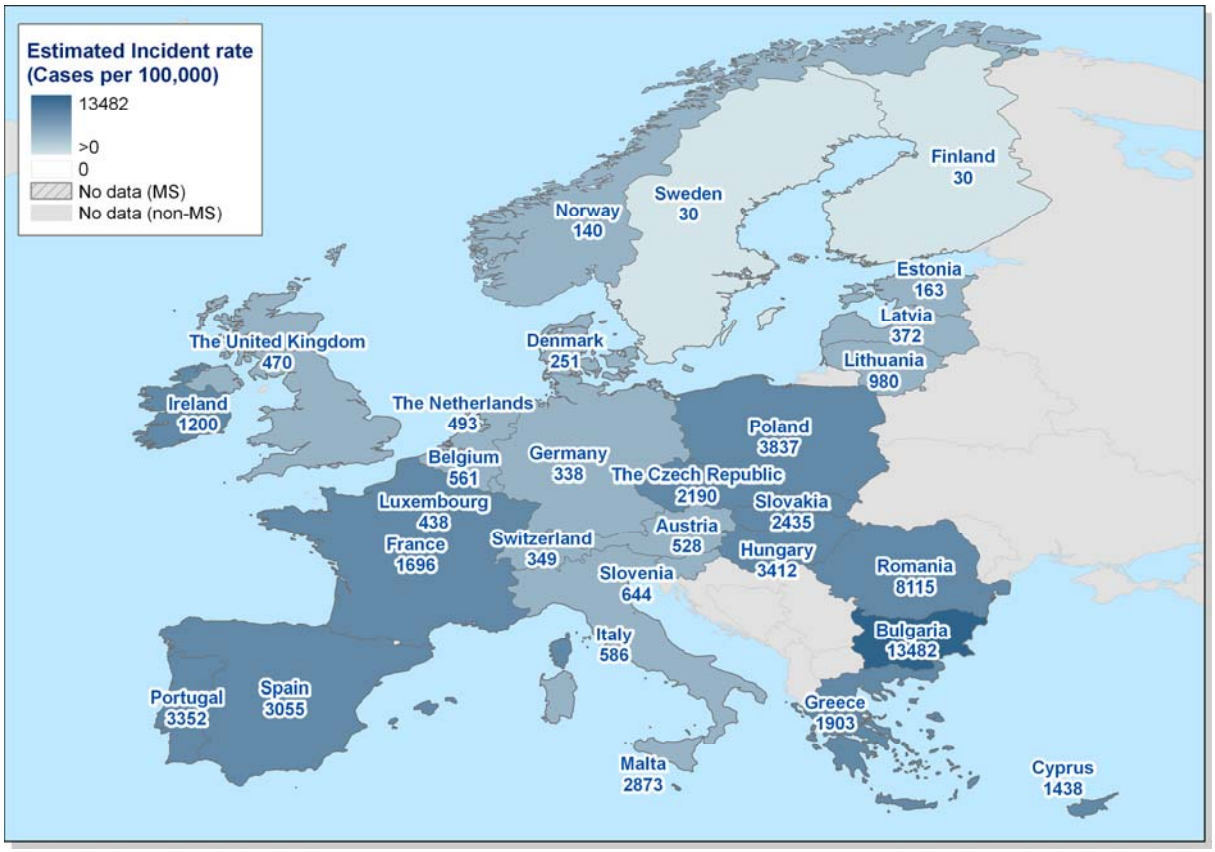

Figure 1: Estimated true incidence rate of human campylobacteriosis in the EU27

The risks to Swedish travellers in the period 2005-2009 were compared with those reported previously by Ekdahl and Giesecke (2004) for the period 1997-2003, see Figure 2. Estimates were highly correlated ($p << 0.001$), and the risks in the 2005-2009 period were similar to those reported before (linear regression model forced through the origin, regression coefficient 0.91).

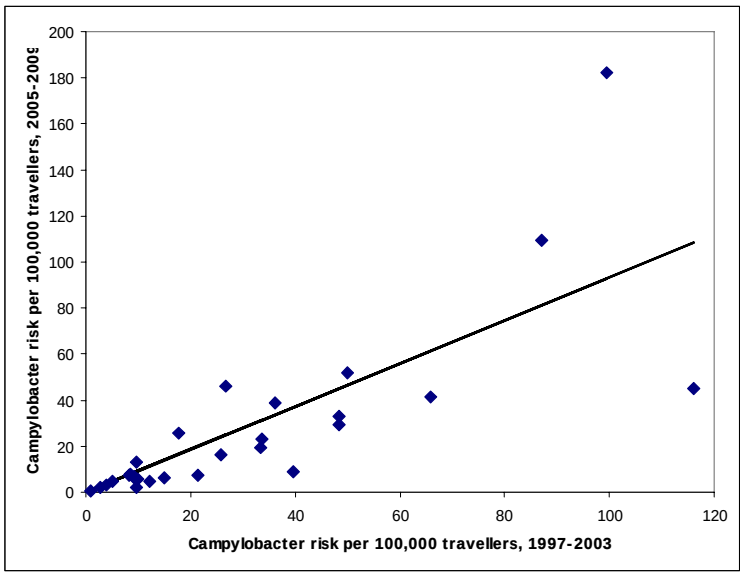

Figure 2: Comparison of campylobacteriosis risks to Swedish travellers in two time periods

Disease incidence per MS was also compared to risks associated with broiler meat as estimated in Figure 3. Bulgaria and Romania were deleted from the analysis because of high leverage. Although there was considerable scatter in the data, a significant correlation ($p = 0.02$) was found between the two risk estimates. This would imply that in countries with a high prevalence and concentration of *Campylobacter* on broiler carcasses, there is also a high risk of human campylobacteriosis.

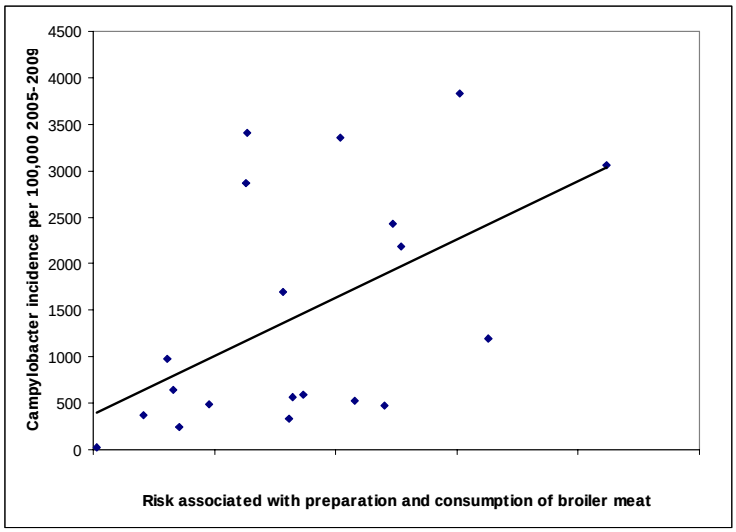

Figure 3: Comparison of estimated true incidence rate of human campylobacteriosis in EU MSs with the risk of campylobacteriosis associated with *Campylobacter* contamination of broiler meat

The data presented above suggest a very high incidence of human campylobacteriosis in the EU (each year, approximately one out of every 50 inhabitants will be affected) and an important contribution of contaminated broiler meat, particularly in high incidence countries. Consequently, considerable public health benefits are expected if *Campylobacter* contamination of broiler carcasses were to be reduced. Nevertheless, the limitations and assumptions in the risk assessment models need to be taken into consideration when interpreting these findings.

The case data are extracted from the Swedish infectious disease surveillance system (SmiNet) and rely on laboratories and physicians reporting diagnosed cases to SMI. Clearly, only a fraction of all cases of illness will be reported. For this model, mainly the potential of differential reporting per country should be considered. Both cases and journeys are counted as such, without consideration of the duration of the stay abroad, the purpose of the visit (business or leisure). Day travels are excluded from the data. There are, for example, a very high number of journeys to Denmark and Finland, which may be mainly for business purposes and of short duration (Ekdahl and Giesecke, 2004). Hence, the duration of exposure may be shorter but on the other hand travellers who fall ill will most likely have returned to their home country and their illness is more likely reported in the Swedish public health system when seeking health care. On the other hand, trips to the Mediterranean area may be mainly for leisure purpose and last one or more weeks. Travellers may be exposed for longer time periods, but when ill may have recovered before returning home. It is difficult therefore to predict in which direction biases may occur. The estimated underreporting factor for Finland is less than one, implying that there are fewer cases than actually reported, which is highly unlikely. This might indicate that for - presumably - short-term visits, the risks to travellers may be underestimated. Further biases may be introduced by seasonal travel patterns. It is likely that most travels to the Mediterranean take place in summer, when the prevalence of *Campylobacter* in animals and food is highest. Health-seeking behaviour of travellers or medical decisions about stool cultures may be affected by the country of destination.

A second important assumption is that relative risks to Swedish travellers are predictive of risks for the local population. This assumption ignores any potential effects, e.g. of acquired immunity, and differences in eating habits between visitors and local residents, as well as differences between strains circulating in different parts of Europe. It is currently not possible to estimate the magnitude or even the direction of these biases. A detailed discussion of potential biases in the data is provided by Ekdahl and Giesecke (2004).

B. EFFECTIVENESS OF DECONTAMINATION TREATMENTS IN REDUCING CAMPYLOBACTER CONCENTRATIONS ON CHICKEN CARCASSES

Chemical decontamination

In some countries, **chlorine**, as **hypochlorite**, has traditionally been used at levels of 50 ppm and higher in the water used during poultry processing, including in the water for immersion chilling. Currently, in the EU, water used for this purpose must be potable, and so does not normally contain more than about 5 ppm chlorine. It is generally agreed that hypochlorite, even at 50 ppm has little effect on bacteria such as *Salmonella* and *Campylobacter* which are attached to the carcass, but that it is effective for inactivating any that are rinsed off or transferred to metal processing equipment, and thus chlorine reduces cross-contamination, particularly during immersion chilling (Bashor *et al.*, 2004; Fabrizio *et al.*, 2002; Li *et al.*, 2002; Mead *et al.*, 1975; Mead *et al.*, 1989). Use of washes at various points during poultry processing, with or without antimicrobials, has generally been found to be beneficial, probably because microbes are more easily detached if carcasses are rinsed immediately after contamination has occurred (Mead *et al.*, 1989). The study by Stopforth *et al.* (2007), in three different processing plants, showed that multiple sequential interventions some of which involved chemical decontaminants, all had a beneficial effect in reducing total counts, *Enterobacteriaceae* and *Salmonella*. Berrang and Bailey (2009) found that multiple washes, with water containing about 40 ppm chlorine, at five different points during processing reduced numbers of *Campylobacter* on carcasses significantly, although the effect of individual washes was not significant. However, Northcutt et al (2005) found that adding chlorine and/or elevating the water temperature during spray washing in an inside-outside bird washer did not enhance the removal of inoculated *Campylobacter* (or other bacteria) from broiler carcasses. The evidence currently available does not indicate that chlorine as hypochlorite would be an effective treatment.

Electrolyzed oxidizing (EO) water is produced by applying an electrical current to sodium chloride solutions and separating the fractions into acidic (anode) and basic (cathode) components. Acidified EO water contains hypochlorous acid (HClO; active ingredient in chlorine) and small amounts of hydrochloric acid, hydrogen peroxide, ozone, and chlorine oxides (Park *et al.*, 2002). The antimicrobial properties of acidified EO result from the synergistic effects of the low pH, high oxidation reduction potential (ORP), and high chlorine content of the water (Northcutt *et al.*, 2007). There are a limited number of reports concerning EO water, and these indicate that dipping seems to be more effective than spraying (Fabrizio *et al.*, 2002). No studies in commercial plants with naturally-contaminated carcasses have been published. Kim *et al.* (2005) compared the efficacy of water alone, acidic EO water and HClO water (both with 40 ppm chlorine,) as a dip or by spraying to reduce numbers of inoculated *Campylobacter* on carcasses. Dipping in EO water or HClO (four litres per carcass) both reduced numbers by about 1 $\log_{10}$ cycle more than water alone. Spraying 6 litres of EO water or HClO per carcass had no effect on numbers of *Campylobacter*. However this study did not indicate the number of replicates or the variability of the results. Also this study used inoculated carcasses, and possibly overestimated the effect of the EO water. In a laboratory experiment, Park *et al.* (2002) inoculated chicken wings with a 6-strain mixture of *C. jejuni.* 50 g of chicken wings was added to 500 ml acidic EO water or chlorinated water (equivalent to 15 litres of rinse fluid to one 1.5 kg carcass), both with 25 ppm Cl_2, (control deionized water) shaking for 10 or 30 min at 23C or 4C. Numbers on the wings were reduced by about 1.5 $\log_{10}$ irrespective of temperature, time or whether EO or chlorine was used. No viable *Campylobacter* were detected in the solutions after the wings had been treated. The study by Northcutt et al (2007) inoculated carcasses with *Campylobacter* and treated them in an inside-outside washer with EO water or chlorine, both with 50 ppm Cl_2 for 5, 10 or 15 sec. However, no water control was used. Reductions of 1.5-2.0 $\log_{10}$ were reported for both chemicals. Treatment lasting for 10 sec was optimal. We do not consider that any published reports convincingly demonstrate that EO water would significantly reduce numbers of *Campylobacter* on carcasses.

Chlorine dioxide has been reported to be more effective than hypochlorite because it is less easily inactivated by organic matter. **Aqueous chlorine dioxide (ClO_2)** is a powerful oxidising and

sanitizing agent, with a broad biocidal activity against bacteria, yeast, viruses, fungi and protozoa. It is a yellow-greenish gas, which is highly soluble in water, but, unlike chlorine, ClO_2 does not react with water. The oxidation capacity of ClO_2 is determined by the oxidation number of the chlorine atom, which is +4, and it can accept 5 electrons when completely reduced to chloride ion. By contrast to chlorine, which reacts via oxidation and electrophilic substitution, ClO_2 only reacts by oxidation, therefore it produces low or very low levels of organochlorine compounds (Richardson *et al.*, 2000). The antimicrobial activity of ClO_2 is related to the loss of membrane permeability control and to non-specific oxidative damage of the outer membrane leading to the destruction of the trans-membrane ionic gradient (Berg *et al.*, 1986). The denaturation of constituent proteins is critical to cellular integrity and function, through the covalent oxidative modification of tryptophan and tyrosine residues, which are also found to be implicated in the lethal activity of ClO_2 (Ogata, 2007). ClO_2 has mostly been investigated as a decontaminating agent for treating fresh produce (Gomez-Lopez *et al.*, 2009) and there are few studies on effectiveness of ClO_2 for the decontamination of fish, meat or poultry. Bolder *et al.* (2007) evaluated the efficiency of ClO_2 against *C. jejuni* in a commercial poultry plant, and obtained reductions of approximately 0.7 $\log_{10}$ with 4.25 ppm ClO_2, whereas water treatment reduced numbers by about 0.35 $\log_{10}$. The treatment was applied via the inside–outside carcass wash, spraying the carcasses for an unspecified time. It is possible that extension of contact time, application of higher concentration, or application through a dipping system would improve the effectiveness of this decontamination agent. However, a study by Vandekinderen *et al.* (2009) using gaseous ClO_2 and studying its effect on various microbes, but not *Campylobacter*, found that it was readily inactivated by protein and fat. Hong *et al.* (2007) reported that dipping inoculated chicken portions for 10 min in solutions of ClO2 at 50 or 100 ppm reduced numbers of *Campylobacter* by 0.99~1.21 $\log_{10}$ cycles. However, no indication was given of the volume of solution per portion, neither did they describe adequately their method of sampling, nor did they confirm the identity of the colonies that they counted on Brucella agar containing blood but no selective agents. Corry *et al.* (2008), working with naturally contaminated carcasses, found that an aqueous ClO_2 spray had no significant effect on numbers of *Campylobacter*.

Kemp *et al.* (2001; 2000) studied the effect of **Acidified sodium chlorite (ASC)** on naturally contaminated carcasses. In their study carried out in five different commercial abattoirs and published in 2001, carcasses visibly contaminated with faeces were either taken off line and specially treated in the way normally used in the USA or treated with an ASC spray (15 sec, 1,200 ppm) immediately after the inside-outside (IO) washer. Examination was by carcass rinse. The 144 carcasses tested after ASC treatment, had 1.75 $\log_{10}$ units lower *Campylobacter* count compared to the 69 reprocessed off-line. Reductions on carcasses not visibly contaminated were stated to be similar and yielding lower final pre-chill levels. In their study published in 2000, naturally contaminated whole carcasses were treated with 1,200 ppm ASC, either by dipping for 5 sec (with agitation, five carcasses per 18.9 l) or by spraying (150 ml in 15 sec). Controls were sprayed with or dipped in water. Numbers of TVC, *E. coli* and coliforms (but not *Campylobacter*) were enumerated using whole carcass rinse. Dipping was more effective than spraying. Reductions in coliforms of 0.93 $\log_{10}$ units by dipping and 0.52 $\log_{10}$ units by spraying were observed compared to using water. A pre-treatment rinse with water was found to improve the effect of the ASC treatment. Schneider *et al.* (2002) reported similar results but did not enumerate *Campylobacter*. In a study enumerating naturally occurring *Campylobacter* on carcasses taken off the line, Oyarzabal *et al.* (2004) treated 80 carcasses with ASC after chilling at 600-800 ppm by dipping for 15 sec. No details were given concerning the ratio of solution to carcasses. Reduction of about 1 $\log_{10}$ unit was achieved. A similar study by Bashor *et al.* (2004) treated 30 naturally contaminated carcasses after the IOBW (ASC 1200ppm spray for 15 sec) and obtained 1.26 $\log_{10}$ units reduction in counts of *Campylobacter*. However, Bolder et al (2007) in a similar study on a commercial line, but using the IOBW to spray with 1200 ppm ASC, reduced numbers of *Campylobacter* by only about 0.5 $\log_{10}$ cycles compared with water (Bolder *et al.* 2007), which might be related to the higher level of organic matter present before rather than after the IOBW. Corry *et al.*(2008) found that ASC (Sanova™ at 1000ppm) as a spray reduced numbers of *Campylobacter* on naturally-contaminated carcasses (taken off the line before the IOBW) by 0.5-1 $\log_{10}$.

Further investigations of ASC as a decontaminant, including the effect of rinsing and the effect on survival of *Campylobacter* during shelf-life could usefully be undertaken. The effect could be best if applied after the IOBW.

Peracetic or peroxyacetic acid (PA) is a strong oxidising agent with a wide spectrum of antimicrobial activity. Commercially it is available as a mixture containing acetic acid, hydrogen peroxide, peracetic acid and water. The antimicrobial activity is associated with damage to DNA and lipids, denaturation of cellular enzymes and proteins, disturbed cell membrane permeability, and interference with protein synthesis through reactions with sulfhydryl, sulfide, and disulfide-containing amino-acids and nucleotides (Kitis, 2004). Bauermeister *et al.* (2008a) inoculated chilled broiler carcasses and then submerged them for 1 h at 4°C in buckets of peracetic acid (two carcasses per 9 litres - no mention of agitation) 0.02% (200 ppm) PA gave ~1 $\log_{10}$ unit better reduction in numbers of *Campylobacter* compared to 0.003% (30 ppm) chlorine (no water control was used). Lower concentrations of peracetic acid (0.0025% and 0.01%) produced lower reductions which were not significantly different from reductions obtained with 0.003% chlorine. Further investigations (Bauermeister *et al.*, 2008b) carried out with naturally-contaminated carcasses in a commercial abattoir, using a commercially-available mixture of peracetic acid and hydrogen peroxide at 85 ppm added to the final chill tank, demonstrated that prevalence of *Campylobacter*-positive carcasses was reduced by 43%, compared to a reduction of 13% using 30 ppm chlorine (numbers of *Campylobacter* were not determined). Chantarapanont *et al.* (2004) investigated the effect of peracetic acid against *C. jejuni* inoculated on chicken skin. They found that dipping in 40 ppm peracetic acid reduced numbers by 0.31 and 0.75 $\log_{10}$ units after 2 and 15 min, respectively. Peracetic acid at 100 ppm resulted in 0.68 and 1.05 $\log_{10}$ reductions, after 2 and 15 min, respectively. However, no water controls were used. Corry *et al.* (2008) found that PAA (commercial product containing 400 ppm peroxyacetic acid, 1600 ppm H_2O_2 and 800 ppm acetic acid) sprayed on naturally contaminated carcasses taken off the line before the IOBW had little more effect on numbers of *Campylobacter* than water.

Trisodium phosphate (TSP) TSP is a highly alkaline product used at 10 – 12% of the hydrated compound, with a pH about 12. A study by Slavik *et al.* (1994) took naturally contaminated carcasses after they had been chilled and dipped them into 10% TSP for 15 sec at 10°C or 50°C. Treatment at 10°C had no significant effect, but treatment at 50°C reduced numbers of *Campylobacter* by 1.2 - 1.5 $\log_{10}$ cycles. Rinsing with water after treatment negated the effect. Similar results were obtained by Whyte *et al.* (2001b), taking naturally contaminated carcasses immediately before chilling and dipping 30 carcasses in 40 litres of 10% TSP at 20°C. Riedel *et al.* (2009) dipped inoculated pieces of skin into 10% TSP for 15 sec and found that numbers were reduced by about 1 $\log_{10}$ cycle compared to dipping in water. Extending the treatment to 5 min improved the effect by about another 0.5 $\log_{10}$ cycle, while the reduction after storage for 24 h at 4°C increased by another $\log_{10}$ cycle. Bashor *et al.* (2004) found that 10% TSP applied as a spray reduced *Campylobacter* numbers on naturally contaminated carcasses in four commercial abattoirs by an extra 1.03 to 1.26 $\log_{10}$ over the reduction achieved with water sprays only. Corry *et al.* (2008) found that 12% TSP sprayed onto naturally contaminated carcasses reduced counts of *Campylobacter* by approximately 0.5 $\log_{10}$ compared to water sprays.

Weak **organic acids** (including lactic, acetic) are frequently used as an inexpensive and effective intervention to reduce numbers and prevalence of bacterial pathogens on food products. Of all organic acids evaluated, acetic, and especially lactic, acid have been found most acceptable. The antimicrobial activity of organic acids is based on the ability of their undissociated form to penetrate through the cell membrane and to dissociate inside the cell, decreasing the intracellular pH value, thus disrupting homeostasis which is essential for the control of ATP synthesis, RNA and protein synthesis, DNA replication and cell growth (Booth, 1985). Besides the decrease in intracellular pH, the perturbation of membrane functions by organic acid molecules might be also responsible for the microbial inactivation. High concentration of anions (due to dissociation) inside the cells might result in an increased osmolarity and consequently the metabolic disruption (Hirshfield *et al.*, 2003).

Lactic acid occurs naturally in meat, is produced by lactic acid bacteria in many fermentations used to preserve foods and has little or no organoleptic effect when used as a surface treatment at 1-2% concentration. Its immediate effect on the microflora is not dramatic, but activity tends to be more marked during subsequent shelf-life. Few investigations have been published on the effect of lactic acid on *Campylobacter* specifically, but many studies have shown that it is useful against pathogens and spoilage flora (Guerrero and Taylor, 1994; Smulders, 1995; Smulders *et al.*, 1986). Buffered lactic acid (pH 3) has been reported to be more effective than an equivalent concentration of lactic acid (Zeitoun and Debevere, 1990), and mixtures of lactic and **acetic acid** more effective than either alone (Corry and Mead, 1996). Some commercially available preparations comprise mixtures of these two acids in combination with other acids, such as citric and ascorbic, designed to improve colour stability (Corry and Mead, 1996).

Riedel *et al.* (2009) used formic and lactic acids to inactivate *C. jejuni* inoculated on chicken skin. The treatment consisted of dipping the inoculated chicken skin into 2.5% lactic acid solution (pH 3.07) or 2% formic acid (pH 2.86) for 1 min at room temperature and determining survivors by rinsing the skin. Reductions were 1.69 and 1.57 $\log_{10}$ CFU/ml of rinse for lactic and formic acid, respectively. The level of reduction increased to 3.87 and >4.2 $\log_{10}$ CFU/ml when a 1 min dip in lactic and formic acid respectively was followed by 24 h storage at 5°C. At the same time, reductions obtained after treatment with sterile water were 0.95 $\log_{10}$ CFU/ml, increasing to only 1.03 $\log_{10}$ CFU/ml after 24 h storage at 5°C. These results clearly showed that the antimicrobial activity of organic acids continues after treatment. Similar results were obtained by Cosansu and Ayhan (2010) who found that 3% acetic acid or 2 % lactic acid reduced numbers of *C. jejuni* on chicken legs or breasts (with skin) by 1 – 2 $\log_{10}$ cycles compared with water as control, and then storage of breast at 4°C reduced *Campylobacter* counts further by comparison with controls.

Results of a study by Zhao and Doyle (2006) revealed that with a suspension of *C. jejuni*, 1% lactic acid had little effect, but acetic acid at 0.5% reduced numbers by >5 $\log_{10}$ cycles within 2 min. Using inoculated chicken wings,15 sec exposure to 2% acetic acid (pH 2.6) at 4°C induced 1.4 $\log_{10}$ CFU/g reduction, while a commercial mixture of acidic calcium sulphate, lactic acid, ethanol, sodium dodecyl sulphate and polypropylene glycol gave a 5-$\log_{10}$ cycle reduction in 15 sec at 4°C. Bolder *et al.* (2007) added 2% buffered lactic acid to the final IOBW in a commercial poultry slaughter plant. The reduction in numbers of *C. jejuni* on naturally contaminated carcasses was approximately 0.8 $\log_{10}$, whereas water treatment produced a 0.35 $\log_{10}$ reduction, revealing only a minor additional effect of organic acid treatment in comparison with water. However, the effect on numbers of *Campylobacter* during subsequent shelf-life was not investigated. A combination of 0.5% benzoic acid/0.5% lactic acid solution (pH 2.64) was applied on chicken wings at 4°C for 30 min by Hwang and Beuchat (1995). This treatment resulted in 1.8 $\log_{10}$ CFU/g reduction in *C. jejuni*, while water treatment reduced numbers by 0.7 $\log_{10}$ CFU/g. In a study by Ellerbroek *et al.* (2007), dipping and spraying of artificially contaminated chicken carcasses with high lactic acid concentrations were compared. Dipping or spraying (at 10°C) with 10% lactic acid for 15 sec did not alter the *Campylobacter* count significantly, but 15% lactic acid (at 30°C) significantly reduced *Campylobacter* count by 0.8 $\log_{10}$ for spraying and 1.5 $\log_{10}$ for dipping. In summary, the only study with a high degree of evidence for the effectiveness of lactic acid is that of Bolder *et al.*(2007), who found that 2% buffered lactic acid in the IOBW reduced numbers of *Campylobacter* by 0.35 $\log_{10}$ cycles.

Physical decontamination

Freezing is a well known method for preservation of foods. The formation of ice crystals will penetrate and kill a large part of the *Campylobacter* present on the meat. However, a fraction of the population may survive or be sub-lethally injured (Georgsson *et al.*, 2006b; Jasson *et al.*, 2007).

Several studies have been published on the effect of freezing, but only a few on an industrial scale looking at naturally contaminated meat. Rosenquist *et al.* (2006) examined the immediate reduction after industrial freezing of naturally contaminated carcasses. They obtained a mean $\log_{10}$ reduction of 1.44 $\log_{10}$ CFU. A similar result was obtained on naturally contaminated carcasses in an Icelandic

study, which showed a reduction of 0.91 $\log_{10}$ immediately after freezing (Georgsson *et al.*, 2006a). There is evidence, though, that the decline in numbers continues during frozen storage. Georgsson et al (2006a) found that levels of *Campylobacter* on carcasses were reduced by 1.77 $\log_{10}$ after 31 days of frozen storage, and remained relatively constant during the days 31–220 of frozen storage. Sandberg *et al.* (2005) also estimated the effect of time of frozen storage on the number of *Campylobacter* on naturally contaminated broiler carcasses. After 3 weeks of frozen storage, they obtained a mean reduction of 2.18 $\log_{10}$ units. Only a marginal extra effect was observed when extending the storage time to 5 weeks. Based on these studies it seems reasonable to conclude that frozen storage for a few days will result in a decrease in *Campylobacter* counts of approximately 1 $\log_{10}$ unit and that storage for 3 weeks will give a total reduction of approximately 2 $\log_{10}$ units. Further storage will only lead to minor further reduction, i.e. the optimum benefit from freezing is obtained if the chicken is stored frozen for 3-4 weeks before consumption.

Due to the documented effect of freezing on numbers of *Campylobacter*, freezing of broiler meat has been implemented as control measure in Iceland (Georgsson *et al.*, 2006a), Norway (Hofshagen and Kruse, 2005) and Denmark (Rosenquist *et al.*, 2006).

Crust-freezing is a technique for rapid chilling of meat and has also been found to accelerate post-mortem 'maturation' to produce tender meat with reduced requirement for chill storage prior to portioning (Kennedy and Miller, 2004). The technique is based on rapid ice crystallization within the meat surface, forming a thin frozen crust followed by temperature equalization/thawing. There are various methods to obtain crust-freezing, e.g. cryogenic freezing (using cryogens N_2 or CO_2) or impingement freezing (using cold high velocity air).

There is little information concerning the effect of crust freezing on *Campylobacter*. One study has investigated the effect on an industrial scale on skinless breast fillets naturally contaminated with *Campylobacter*. The fillets were treated in a commercial continuous CO_2, belt freezer, which transported the fillets into a -freezing zone at -55 ˚C. Fillets were crust-frozen individually and reached an outer surface temperature of approximately -1 ˚C after treatment. The mean reduction obtained was 0.42 $\log_{10}$ units (Boysen and Rosenquist, 2009). A similar reduction (0.4 $\log_{10}$ units) was obtained in a study by Mead (Mead, 2004). Due to the fact that this study was carried out on a pilot scale with inoculated strains, the results are not given as high priority as the study by Boysen (2009).

That crust freezing can reduce numbers of *Campylobacter* is supported by a study examining crust-freezing on *C. jejuni* inoculated onto the breast skin of carcasses (James *et al.*, 2007) using a pilot chilling chamber and the time-temperature combination -35 ºC for 23 min, -10 ºC for 70 min then + 15 ºC for 30 min. The reductions obtained in two different series were 1.78 $\log_{10}$ and 1.00 $\log_{10}$ units. However, these data refer only to inoculated breast skin on carcasses, and might over-estimate the effect for other parts, e.g. the body cavity of naturally contaminated carcasses. It is also known that studies using inoculated strains usually overestimate the reducing effect. Another study using inoculated carcasses estimated the effect to be 0.9 $\log_{10}$ (Corry *et al.*, 2003). Therefore, the reduction that can be obtained with inoculated strains is probably closer to 1 $\log_{10}$ unit.

Heat treatment is another way of killing microorganisms. C**ooking** is a well-known method for reducing levels of pathogenic bacteria in food. Based on the literature, proper cooking will kill the *Campylobacter* present on broiler meat as *Campylobacter* species are very sensitive to heat (ICMSF, 1996). Whyte *et al.* (2006) report that naturally occurring *Campylobacter* spp were inactivated in liver if the internal temperature reached a maximum of 70–80 °C for 2–3 min. Sampers *et al.* (2010) found that when internal temperatures reached 57.5 °C, *Campylobacter* numbers dropped below detectable levels (<10 CFU/g). Further on, a study by Bergsma *et al.* (2007) predicts that recommended cooking times suffice to adequately reduce *C. jejuni* counts by frying chicken breast fillets. Based on these studies it is concluded that proper heat treatment reduces numbers of *Campylobacter* by more than 6 $\log_{10}$ units.

Heating the surface of the meat to reduce numbers of *Campylobacter* leaving the meat without a heat treated appearance has been investigated as decontamination technique. Effects on *Campylobacter* counts by applying steam, hot water or steam simultaneously with ultrasound have been reported. Several studies have looked at **hot water** for decontamination of poultry, but only few of these have been done on an industrial scale. Purnell *et al.* (2004) used 'hot wash' treatments to reduce the number of pathogenic and spoilage bacteria on naturally contaminated carcasses fresh from the production line. An experimental in-line processing unit for poultry carcasses using hot water immersion was developed for the study. They found that treatment at 75 °C for 30 s significantly reduced the numbers *Campylobacter* (1.6 $\log_{10}$) but the chicken skin tended to tear during trussing. A treatment of 70 °C for 40 s had no effect on *Campylobacter*, but a treatment of 70 °C for 40 s followed by a 12–15 °C, 13 s spray chill treatment, had no detrimental effect on the chicken skin, and the numbers of all groups enumerated remained significantly lower than the controls for 8 days under typical chilled storage conditions (from 0.13 $\log_{10}$ in one trial to >1.36 in another). It was not clear whether the effect was due to the heat treatment or the chilling, as there was no control showing the effect of the chilling method. Berrang *et al.* (2000) examined the effect of a second scald applied after defeathering on microbial levels recovered from carcass rinses. Four treatments were evaluated: 1) immersion at 60 °C for 28 s 30 min after defeathering, 2) immersion at 60 °C for 28 s immediately after defeathering, 3) spray at 73 °C for 20 s 30 min after defeathering, and 4) spray at 71 °C for 20 s immediately after defeathering. However, neither the immersion nor the second spray scald treatments lowered the *Campylobacter* counts. Reductions of *Campylobacter* counts have, though, been obtained with naturally occurring *Campylobacter* on carcasses using immersion in hot water at 75-85 °C in laboratory scale trials (Whyte *et al.*, 2003). A moderate degree of deterioration in physical appearance of broiler skin due to immersion treatments was obtained for temperatures of 75°C for 20 s; 80°C for 10 s and 85°C for 10 s. The reductions obtained for these combinations of time and temperature were 0.64, 0.27 and 0.43 $\log_{10}$ units.

Corry *et al.* (2007) evaluated in laboratory trials the effect of hot water on poultry carcasses artificially inoculated with *C. jejuni* on breast skin. Inoculated carcasses were dipped in water at 70 ˚C for 40 s, 75 ˚C for 30 s and 80 ˚C for 20 s. These treatments gave reductions of 0.98, 1.66 and 1.27 $\log_{10}$ units. However, the treatments also resulted in some slight changes in appearance in the treated carcasses, i.e. at cut edges and exposed muscle of the carcasses and some shrinking of the skin, but the effect was much less marked on portions cut from the treated carcasses. Further work with naturally-contaminated carcasses taken straight off the line and treated at 80°C for 20 sec showed reductions in numbers of *Campylobacter* of 1 – 1.5 $\log_{10}$ cycles (Corry *et al.*, 2006).

In conclusion, hot water treatment of broiler carcasses may result in reductions in *Campylobacter* counts varying from 0.27 and 1.5 $\log_{10}$ units.

Heat treatment using **steam** has also been investigated in a few studies. (Whyte *et al.*, 2003) investigated naturally contaminated carcasses in a steam pasteurization unit installed in-line in a beef abattoir. A moderate degree of deterioration in physical appearance of broiler skin was observed after treatment at 90°C for 12 s which resulted in a reduction of 0.46 $\log_{10}$ units in numbers of *Campylobacter*. It was found that longer carcass exposure times caused more damage to the skin.

James *et al.* (2007) investigated the effect of steam on the number of inoculated *C. jejuni*. Whole chicken carcasses were treated in a pilot steam cabinet. The treatments caused the skin to shrink and change colour. Acceptable changes were obtained at treatment times less than 12 s. Treatment for 10 s resulted in a reduction of the inoculated strain of 1.8 $\log_{10}$ units. Because this study was carried out on a pilot scale using an inoculated strain, the results obtained by Whyte and co-workers are given higher priority and it is concluded that steam treatment may cause a reduction of around 0.5 $\log_{10}$ units.

Steam-ultrasound is a decontamination technique under development. It is based on simultaneous treatment of the meat surface with steam and ultrasound. The hypothesis behind the method is that ultrasound enhances the killing effect of hot steam by efficiently removing the protective air on the meat surface allowing the steam more efficiently to reach the bacteria in the microstructure and

cavities of the meat surface. The combination should therefore optimize steam treatment and result in a shorter treatment time at high temperature.

The reductions in counts of *Campylobacter* on carcasses obtained by steam-ultrasound treatment have varied largely. On-line experiments with the first proof-of-concept equipment resulted in reductions of 2-3 $\log_{10}$ units of naturally occurring *Campylobacter* spp. (Boysen and Rosenquist, 2009). However, in these experiments the appearance of the carcass surface was not satisfactory. The equipment was therefore optimized and the treatment time reduced. Preliminary results from this improved equipment have not been able to reach the same reduction as seen in the initial experiments. The technique has shown promising results, but more studies are needed with the on-line equipment to demonstrate the actual effect in industrial scale processing.

It is well established that ionizing **irradiation** is effective in killing enteric pathogens in fresh foods such as broiler meat. Different technologies can be used for the irradiation of food. According to the Codex General Standard for Irradiated foods[17] , ionizing radiation can be obtained by gamma rays, X-rays or electron beams. While gamma rays are produced from a radioactive source, X-rays and electron beams are produced by specific equipment converting other energy sources, such as electric current, without the involvement of any radioactive substance. However, none of these methods have energy levels sufficient to induce radioactivity in the irradiated food when used at the doses established by the Codex Standard. Irradiation of food at EU level is regulated by two pieces of legislation; Directive 1999/2/EC[18] and Directive 1999/3/EC[19] . Directive 1999/3/EC contains a list of foodstuffs authorized for irradiation treatment and the doses allowed. So far, only dried aromatic herbs, spices and vegetable seasonings are included in the list. However, irradiation of other foodstuffs including poultry is temporarily permitted in some Member States.

In 1986, the Scientific Commitee on Food evaluated 7 kGy to be acceptable for treatment of poultry (SCF, 1986). A recent opinion from EFSA on irradiation of food, concluded that this dose would be sufficient to give at least a 5-$\log_{10}$ reduction in the number of vegetative pathogens from frozen poultry meat. Lower doses would be sufficient to achieve the same reduction in chilled poultry meat (EFSA 2011). Even doses of 3–5 kGy and 1.5–2.5 kGy are reported as adequate to eliminate *Salmonella* and *Campylobacter* in frozen and chilled poultry, respectively (Corry *et al.*, 1995; Dincer and Baysal, 2004; Farkas, 1998; Hugas and Tsigarida, 2008). In the United States, FDA and USDA have approved irradiation of poultry meat at a maximum dose of 3 kGy to control foodborne pathogens such as *Campylobacter* and *Salmonella* (Keener *et al.*, 2004).Irradiation can be used on pre-packaged chilled or frozen poultry meat, although higher doses of irradiation are required for frozen product. It also has the advantage of inactivating *Campylobacter* below the skin or surface, which do occur in low numbers (Luber and Bartelt, 2007). It could also be used to treat chicken liver. Irradiation using gamma rays is more penetrating than X-rays or electron beams, so the latter might be better for treating poultry meat portions rather than whole carcasses.

Based on these results, it is concluded that irradiation at 3 kGy will decrease *Campylobacter* counts about 3 to 6 $\log_{10}$ units, depending on the temperature of the meat.

In addition to ionizing radiation, electromagnetic waves such as **ultraviolet (UV) light** are also used in the food industry for the disinfection of surfaces of packaging materials or food processing equipment and environment. Although *Campylobacter* is relatively sensitive to UV radiation, its use in poultry and other foods is limited due to its low penetration power which allows microbes to survive when present in shadowed crevices (Corry *et al.*, 1995). No industrial studies have been performed, but a few studies have looked at effects of UV light on inoculated strains of *Campylobacter*. Isohanni and Lyhs (2009) found the maximum reduction of inoculated *C. jejuni* by UV light to be 0.7 $\log_{10}$ units and 0.8 $\log_{10}$ units on broiler meat and on broiler skin, respectively, while on broiler carcasses the

[17] General standard for irradiated foods CODEX STAN 106-1983, REV.1-2003, www.codexalimentarius.net/download/standards/16/CXS_106e.pdf
[18] OJ L 66, 13.3.1999, p. 16–23
[19] OJ L 66, 13.3.1999, p. 24–25

maximum reduction was 0.4 $\log_{10}$ units. A study by Chun *et al.* (2010) indicated that UV-C treatment at 5 kJ/m2 decreased the population of inoculated *C. jejuni* on chicken breasts by 1.26 $\log_{10}$ cycle. Since the effect of UV light has not been documented in industrial scale on naturally contaminated meat, we cannot conclude on the effect of this intervention. This also applies to **high hydrostatic pressure processing (HPP)**.

C. INTERVENTION ANALYSIS USING CAMO

1. INTRODUCTION

Four study countries were selected based on the available data for assessing the effect of intervention scenarios, the range of prevalences and counts on the broiler carcasses.

Table 4: Selection of countries for modelling based on EU baseline survey data

	Country 1 (C1)	Country 2 (C2)	Country 3 (C3)	Country 4 (C4)
Prevalence of *Campylobacter*-colonized batches	low	Medium	medium	high
Level of carcass contamination	low	High	medium	medium

C2 has a relatively high proportion of outdoor flocks

Input data for the current state other than those from the EU baseline survey in these countries were delivered by ad-hoc WG and BIOHAZ Panel. A selection of interventions was applied in at least one of the four countries, unless otherwise stated, using CAMO.

Table 5: Input table of interventions

Section	Intervention	Target	Model
3.1	Target indoor prevalence	Between-flock prevalence(BFP)[1]	Calculation of new prevalence based on targets
3.2	Biosecurity in C4	Between-flock prevalence	Implemented in model as the beta coefficient that corresponds to a hazard ratio of 0.40, (0.15, 1.09) p=0.06 (Gibbens *et al.*, 2001)
3.3	Fly screens in C3	Between-flock prevalence	Implemented in model as a slaughter age-weighted k-factor[2] of 0.47 (21 days of slaughter age), 0.15 (28 days of slaughter age) and 0.10 (35 days of slaughter age) (Hald *et al.*, 2007)
3.4	Discontinued thinning	Between-flock prevalence	BFP estimate OR = 1.74, implemented in model as regression coefficient (0.5521) (EFSA, 2010a)
3.5	Reduction of slaughter age	Between-flock prevalence	BFP estimate OR = 1.98 per 10 days increase, implemented in model as regression coefficient (0.06742) (EFSA, 2010a)
3.6	Reducing colonization	*Campylobacter* concentration in caecal material[1]	Reduction of mean concentration on carcass
3.7	Decontamination	*Campylobacter* concentration on carcass	Reduction of mean concentration on carcass
3.8	Scheduled slaughter	Proportion of positive flocks to which decontamination is applied	Reduction of mean concentration on carcass in targeted flocks

[1] when the prevalence is the target, the "dose response" model has no impact, when the concentration is the target the choice of the "dose response" model is crucial.

[2] k-factor $k_{i+1} = \frac{p_{i+1} * (1 - p_i)}{p_i * (1 - p_{i+1})}$

2. Input parameters

The following information was included in the model. Various inputs* were derived from the EU baseline survey (EFSA, 2010):

- The **percentage of indoor farms*** as a percentage of the total number of broiler farms was 71% in C2, 100% in C3, 100% in C1, and 94% in C4.

- Thinning was assumed to occur only in indoor broiler flocks. The **percentage of previously thinned indoor flocks*** as a percentage of the number of indoor flocks with known thinning status was 82% in C2, 25% in C3, 3% in C1, and 65% in C4.

- The **mean age of indoor broiler flocks*** was 40.9 days (Standard Deviation (SD)=4.4, min=33, max=79) in C2, 37.7 days (SD=2.0, min=32, max=42) in C3, 32.4 days (SD=2.0, min=28, max=41) in C1, and 39.9 days (SD=5.2, min=28, max=58) in C4.

- The **between-flock prevalence** (BFP)*, expressed as the percentage of *Campylobacter* positive broiler batches sampled at the slaughter plant, was 30.27% in C2, 19.19% in C3, 3.28% in C1, and 75.81% in C4. These estimates were obtained not taking into account correlation of observations within the same slaughter-house. This correlation was used to assess BFP in the EU baseline surevy report (EFSA, 2010a).

- The **counts on the broiler carcasses*** (neck and breast skin) after chilling were 2.72 $\log_{10}$ (SD=0.92) CFU of *Campylobacter* in C2, 2.21 $\log_{10}$ (SD=1.10) CFU in C3, 0.94 $\log_{10}$ (SD=0.49) CFU in C1, and 2.36 $\log_{10}$ (SD=1.16) CFU in C4.

The **reporting factor** was set to 4.2% in C2, 30.0% in C3, 42.5% in C1, and 11.4% in C4, as described by Ekdahl and Giesecke (2004). More recent reporting values can be found in Appendix A. , but using these newer figures was observed not to affect the reduction in public health risk if expressed in relative terms, which is the case in this report and this Opinion.

The input parameters for the four study countries are summarized in Table 3.

- Current state of **hygiene/biosecurity*** was deduced from EU baseline survey data. For indoor flocks, the most likely input for current state for hygiene/biosecurity was calculated as the mean of the maximum level of biosecurity achieved by the country (calculated as 100 minus $\text{prevalence}_{\text{winter}}$) and the minimum level of biosecurity achieved (calculated as 100 minus $\text{prevalence}_{\text{summer}}$). The summer prevalence was calculated using data of the flocks sampled during the third quarter in the EU baseline survey (i.e. July, August and September), while for winter prevalence, data was used from the first quarter in the baseline survey (i.e. January, February and March) (see Table 4). For outdoor flocks, the current state of biosecurity was set to 0%.

- Current state for **water treatment of the broiler flocks with organic acids** was gathered using expert opinions by the ad-hoc WG and BIOHAZ Panel. It was assumed that the values were valid for both indoor and outdoor flocks. For C2, the expert opinion for most likely value was 30%. In C3, C1, and C4 this treatment is not applied.

- Current state for the **detection of highly faecally contaminated carcasses** at the slaughter-house. As agreed with the Experts, the detection of highly contaminated carcasses was set to the most likely value of 50% for the four study countries both for indoor and outdoor flocks.

- Current state for **transport, bacteriophages, bacteriocins, vaccination, fly screens, logistic and scheduled slaughter, all decontamination steps before and after the chiller.** The current state values for these model inputs were set to 0% both for indoor and outdoor flocks for the four study countries. As the future state for "Transport" was not assessed, the input

value does not matter. The current state for bacteriophages, bacteriocins, vaccination, fly screens was set to 0% because these are not applied at the moment. Current state for logistic and scheduled slaughter was set to 0%. Also the current state for all decontamination steps before and after the chiller (physical and chemical) was set to 0%. For freezing, it was discussed to set the values to 0% as it was assumed that most-risky meats are not frozen.

Table 6: Input parameters (Most Likely value) for the four study countries(a)

Input	C1		C2		C3		C4	
	Indoor farms	Outdoor farms	Indoor farms	Outdoor farms	Indoor farms	Outdoor farms	Indoor farms	Outdoor farms
Hygiene/biosecurity	96	0	73	0	78	0	24	0
Water treatment with organic acids	0	0	30	30	0	0	0	0
Detection of highly faecally contaminated carcasses	50	50	50	50	50	50	50	50

(a): All the other input parameters were set = 0

Table 7: Seasonal between-flock-prevalence (BFP) of *Campylobacter* positivity of broiler batches at the slaughter plant[(a)]

Country	Winter			Summer		
	s	n	BFP (%)	s	n	BFP (%)
C1	0	101	0	8	95	8
C2	26	108	24	18	59	31
C3	5	85	6	38	99	38
C4	68	102	67	85	99	86

(a): s = number of positive samples; n = total number of samples

3. Intervention analysis

3.1. Evaluation of targets for prevalence (CamPrev)

3.1.1. Implementation

- 25 EU Member states;
- We calculated the risk reduction if all the MS have a BFP lower than a certain value P (target 50%, 25%, 10%, 5%, 1% and 0%) and assuming that no control option is undertaken in the MS where the current BFP (Pc) is lower than the target prevalence P. The new prevalence would be P_{new} = min(Pc, P) and the risk reduction=(P_{new}-Pc)/Pc.
- To calculate the EU averaged risk reduction, the new overall BFP was weighted using the broiler production weight. The EU risk reduction was calculated using this new overall BFP.

3.1.2. Result

Table 5 gives an overview of the relative reduction in human cases if certain values of BFP (below the current BFP) would be achieved.

The EU averaged risk reduction are 29.3%, 61.6%, 84.4%, 92.1% and 98.4% when targets of 50%, 25%, 10%, 5% and 1% respectively are achieved, including indoor and outdoor flocks. To achieve an

EU averaged risk reduction of 25%, 50% and 90%, the BFP targets should be set to 54%, 34% and 6%.

Table 8: Relative reduction in human cases if certain values of between-flock prevalence (BFP) were achieved

Country		Current BFP[a]	Risk reduction if BFP would be reduced to					
			50%	**25%**	**10%**	**5%**	**1%**	**0%**
Austria	AT	47.8%	0.0%	47.7%	79.1%	89.5%	97.9%	100.0%
Belgium	BE	30.3%	0.0%	17.4%	67.0%	83.5%	96.7%	100.0%
Bulgaria	BG	33.1%	0.0%	24.5%	69.8%	84.9%	97.0%	100.0%
Cyprus	CY	31.7%	0.0%	21.2%	68.5%	84.2%	96.8%	100.0%
Czech Republic	CZ	61.1%	18.2%	59.1%	83.6%	91.8%	98.4%	100.0%
Denmark	DK	19.2%	0.0%	0.0%	47.9%	73.9%	94.8%	100.0%
Estonia	EE	2.0%	0.0%	0.0%	0.0%	0.0%	49.0%	100.0%
Finland	FI	4.1%	0.0%	0.0%	0.0%	0.0%	75.8%	100.0%
France	FR	75.1%	33.4%	66.7%	86.7%	93.3%	98.7%	100.0%
Germany	DE	48.6%	0.0%	48.6%	79.4%	89.7%	97.9%	100.0%
Hungary	HU	50.5%	0.9%	50.5%	80.2%	90.1%	98.0%	100.0%
Ireland	IE	80.7%	38.1%	69.0%	87.6%	93.8%	98.8%	100.0%
Italy	IT	63.9%	21.7%	60.9%	84.3%	92.2%	98.4%	100.0%
Latvia	LV	41.0%	0.0%	39.0%	75.6%	87.8%	97.6%	100.0%
Lithuania	LT	42.0%	0.0%	40.4%	76.2%	88.1%	97.6%	100.0%
Malta	MT	97.0%	48.5%	74.2%	89.7%	94.8%	99.0%	100.0%
Poland	PL	79.2%	36.9%	68.4%	87.4%	93.7%	98.7%	100.0%
Portugal	PT	82.9%	39.7%	69.8%	87.9%	94.0%	98.8%	100.0%
Romania	RO	76.5%	34.6%	67.3%	86.9%	93.5%	98.7%	100.0%
Slovakia	SK	70.6%	29.2%	64.6%	85.8%	92.9%	98.6%	100.0%
Slovenia	SI	77.7%	35.7%	67.8%	87.1%	93.6%	98.7%	100.0%
Spain	ES	87.7%	43.0%	71.5%	88.6%	94.3%	98.9%	100.0%
Sweden	SE	12.4%	0.0%	0.0%	19.6%	59.8%	92.0%	100.0%
The Netherlands	NL	24.2%	0.0%	0.0%	58.8%	79.4%	95.9%	100.0%
United Kingdom	UK	75.8%	34.0%	67.0%	86.8%	93.4%	98.7%	100.0%
Weighted EU average			**29.3%**	**61.6%**	**84.4%**	**92.1%**	**98.4%**	**100.0%**
Norway	NO	3.3%	0.0%	0.0%	0.0%	0.0%	69.5%	100.0%
Switzerland	CH	59.5%	15.9%	58.0%	83.2%	91.6%	98.3%	100.0%

(EFSA, 2010a)

3.2. Biosecurity in C4

3.2.1. Implementation

- Country: C4
- Use "Hygiene from proportional-hazard survival analysis result" (using the b-value from the model in Table 6, Gibbens *et al.* (2001));
- Run in three different deterministic scenarios for the b-value: median, lower 95% CI, upper 95% CI and applying biosecurity in future state fractions of 100% in indoor flocks only;
- Run in deterministic scenario using the median value and increase the future state fraction of biosecurity gradually from 24% (=current state) to 100% in indoor flocks only.

3.2.2. Result

The effect of applying biosecurity as practically implemented in the Gibbens study in all indoor flocks in the C4 (instead of the 24% currently) is expected to lead to a relative reduction in human cases of 16.33%. The range of the relative reduction is broad: from 0% to 36.53% (Table 6). A linear increase

is obtained by applying biosecurity in more indoor flocks. The same effect is obtained using the three dose-response models considered.

Table 9: Effect of biosecurity in all the indoor flocks in the C4 on the relative reduction in human cases

Future state	Simple exp/Beta-Poisson/Classic+ Indoor and outdoor flocks	Simple exp Indoor flocks	Simple exp Outdoor flocks
24% (Current state, median)	0.00%	0.00%	0.00%
30% (median)	1.29%	1.37%	0.00%
40% (median)	3.44%	3.66%	0.00%
50% (median)	5.59%	5.94%	0.00%
60% (median)	7.74%	8.23%	0.00%
70% (median)	9.89%	10.51%	0.00%
80% (median)	12.03%	12.80%	0.00%
90% (median)	14.18%	15.09%	0.00%
100% (median)	16.33%	17.37%	0.00%
100% (lower 95% CI)	0.00%	0.00%	0.00%
100% (upper 95% CI)	36.53%	38.85%	0.00%

3.3. Fly screens in C3

3.3.1. Implementation

- Country: C3;
- Effect was applied during 5 months: June-July-August-September and October;
- We changed the future state to 100% and applied the k-factor of 0.47 weighted for age distribution (21 days of slaughter age), 0.15 (28 days of slaughter age) and 0.10 (35 days of slaughter age), from Hald *et al.* (2007);
- Future state fraction was set to 100% for indoor flocks only.

3.3.2. Result

The application of fly screens to all indoor flocks in C3 is estimated to lead to a 60.07% relative reduction in human cases, irrespective of the DR model.

3.4. Discontinued thinning

3.4.1. Implementation

- Countries: C1, C2, C3, and C4;
- The thinning regression coefficient (0.5521) was obtained from the EU baseline survey (EFSA, 2010b) and applies to all countries;
- For outdoor flocks, thinning is set to 0%, both for current and future state;
- For indoor flocks, future state fractions are set to 0%.

3.4.2. Result

The relative reduction in human cases by stopping thinning is greatly influenced by the country considered. It has a minor effect (1.79%) in C1, would reduce 12.60% of the cases in C3 and about a quarter of the cases in C2 and C4 (Table 7). The three dose-response models show the same effect.

Table 10: Effect of stopping thinning indoor flocks (future state 0%) on the relative reduction in human cases for consumers of broiler meat from flocks coming from different production systems

Country	Indoor and outdoor flocks	Indoor flocks	Outdoor flocks
C1	1.79%	1.79%	0.00%
C2	25.22%	35.71%	0.00%
C3	12.60%	12.63%	0.00%
C4	25.07%	26.67%	0.00%

3.5. Slaughter age

3.5.1. Implementation

- Countries: C1, C2, C3, and C4;
- Future state: maximum slaughter age of indoor flocks has been set to 28 days, 35 days and 42 days.

3.5.2. Result

The relative reduction in human cases by reducing the slaughter age of indoor flocks is also greatly influenced by the country considered (Figure 1). Reducing the maximum slaughter age to 42 days has a limited effect for all countries (below 5% reduction in human cases). By reducing the maximum age to 35 days and 28 days respectively, human cases are reduced by 0.6% to 18% and 21% to 43%, respectively (depending on the country). The effect was highest in C2 and C3.

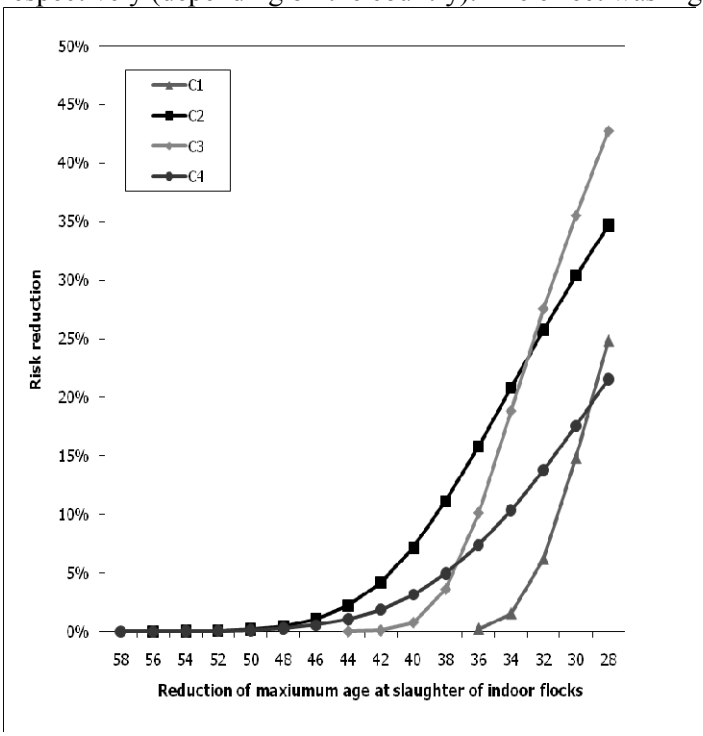

Figure 4: Effect of reducing the slaughter of indoor flocks on the relative reduction in human cases (at country level) for consumers of broiler meat from flocks coming from different production systems

3.6. Reduced colonization

3.6.1. Implementation

- Countries: C1, C2, C3, and C4;
- The effect of a 1, 2, 3, and 6 $\log_{10}$ reduction of *Campylobacter* caecal count of broilers, both for indoor and outdoor flocks, was assessed. Two assumptions were made to translate this reduction into reduced numbers on the carcass (numbers given relate to 3 $\log_{10}$ reductions in shedding):
  - Assumption 1: 3 $\log_{10}$ reductions in caecal counts will result in 3 $\log_{10}$ reduction on carcasses.
  - Assumption 2: 3 $\log_{10}$ reductions in caecal counts will result in 1.92 $\log_{10}$ reduction on carcasses (correlation of 0.64 as obtained by Reich *et al.*, 2008)

3.6.2. Result

Tables 9-11 list the relative reduction in human cases by reducing the caecal counts of *Campylobacter* of the broilers of indoor and outdoor flocks with 1, 2, 3, and 6 $\log_{10}$ units. Considering the Classic+ DR model, the relative reduction in human cases is over 65% for all countries with a reduction of 1 $\log_{10}$, above 91% if the reduction was 2 logs, above 98% with a 3 $\log_{10}$ reduction and 100% for 6 $\log_{10}$. Under assumption 2 relative reductions in human cases are estimated as 48%, 76%, 90% and 100%, respectively. Using the simple exponential and the ,odified Beta-Poisson DR models, relative reductions in human cases are lower than in case of the classic + DR model.

Table 11: Effect of 1, 2, 3 and 6 $\log_{10}$ reduction of *Campylobacter* in caecal contents of broilers, both for indoor and outdoor flocks, using the classic + DR model on the relative reduction in human cases

Red. caecal counts		**1 $\log_{10}$**		**2 $\log_{10}$**		**3 $\log_{10}$**		**6 $\log_{10}$**	
Red. carcass		**1**	**0.64**	**2**	**1.28**	**3**	**1.92**	**6**	**3.84**
Country	C1	83.21%	67.72%	97.51%	90.06%	99.70%	97.06%	100.00%	99.95%
	C2	66.56%	48.59%	91.94%	76.76%	98.61%	90.85%	100.00%	99.75%
	C3	67.04%	49.36%	91.68%	76.96%	98.39%	90.62%	100.00%	99.67%
	C4	65.52%	47.83%	91.01%	75.63%	98.26%	89.88%	100.00%	99.65%

Table 12: Effect of 1, 2, 3 and 6 $\log_{10}$ reduction of *Campylobacter* in caecal contents of broilers, both for indoor and outdoor flocks, using the simple exponential DR model on the relative reduction in human cases

Red. caecal counts		**1 $\log_{10}$**		**2 $\log_{10}$**		**3 $\log_{10}$**		**6 $\log_{10}$**	
Red. carcass		**1**	**0.64**	**2**	**1.28**	**3**	**1.92**	**6**	**3.84**
Country	C1	33.98%	21.75%	67.96%	43.49%	94.57%	65.25%	99.99%	99.07%
	C2	21.18%	13.56%	42.36%	27.12%	63.31%	40.67%	99.30%	79.56%
	C3	23.75%	15.20%	47.34%	30.39%	69.58%	45.48%	99.34%	84.65%
	C4	22.92%	14.68%	45.64%	29.32%	67.17%	43.85%	99.13%	82.35%

Table 13: Effect of 1, 2, 3 and 6 $\log_{10}$ reduction of *Campylobacter* in caecal contents of broilers, both for indoor and outdoor flocks, using the Beta-Poisson DR model on the relative reduction in human cases

Red. caecal counts		**1 $\log_{10}$**		**2 $\log_{10}$**		**3 $\log_{10}$**		**6 $\log_{10}$**	
Red. carcass		**1**	**0.64**	**2**	**1.28**	**3**	**1.92**	**6**	**3.84**
Country	C2	16.11%	9.72%	37.50%	21.56%	62.28%	35.61%	99.43%	80.87%
	C3	20.59%	12.55%	45.72%	27.29%	70.54%	43.63%	99.46%	86.13%
	C1	38.49%	23.57%	76.41%	50.19%	95.84%	74.04%	99.99%	99.29%
	C4	19.22%	11.71%	42.87%	25.48%	67.18%	40.88%	99.29%	83.55%

3.7. Decontaminations treatments in slaughter-house

3.7.1. Implementation

- Countries: C1, C2, C3,and C4;
- Future state set to 100% for both indoor and outdoor flocks;
- Uncertainty and variability in effect estimates were not taken into account.

3.7.2. Result

The relative reduction in human cases by a selected number of decontamination treatments in the slaughter-house are listed in Tables 11-13 for the four study countries.

The reductions are very similar for C2, C3 and C4, but in general higher for C1 (see Figure 2). For all treatment effects (to a lesser extent for irradiation/heat treatment), the relative reductions are higher using the Classic+ DR model compared to the Simple exponential and Beta-Poisson DR model.

Table 14: Effect of decontamination treatments in slaughter-house on the relative reduction in human cases in the four study countries, using the Classic + DR model

Treatment	**Effect ($\log_{10}$ red)**	**C1**	**C2**	**C3**	**C4**
Irradiation/cooking	6.00	100.00%	100.00%	100.00%	100.00%
Short term freezing: Rosenquist *et al.* (2006)	1.44	92.59%	81.35%	81.40%	80.22%
Short term freezing: Sandberg *et al.* (2005)	0.99	82.90%	66.14%	66.63%	65.10%
Short term freezing: Georgsson *et al.* (2006)	0.91	80.17%	62.62%	63.19%	61.63%
Long term freezing: Sandberg *et al.* (2005)	2.18	98.30%	93.98%	93.69%	93.16%
Long term freezing: Georgsson *et al.* (2006)	1.77	96.05%	88.47%	88.30%	87.43%
Lactic acid: Bolder (2007)	0.47	55.72%	37.56%	38.36%	37.01%
Hot water: Corry *et al.* (2006)	1.25	89.49%	75.81%	76.04%	74.68%
Acidified sodium chlorite: Kemp (2001)	1.75	95.90%	88.12%	87.96%	87.07%
Acidified sodium chlorite: Bashor *et al.* (2004)	1.26	89.68%	76.13%	76.35%	75.00%
Trisodium phosphate: Bashor *et al.* (2004)	1.03	84.11%	67.80%	68.25%	66.74%

Table 15: Effect of decontamination treatments in slaughter-house on the relative reduction in human cases in the four study countries, using the Simple exponential DR model[(a)]

Treatment	Effect (log_{10} red)	C1	C2	C3	C4
Irradiation/cooking	6.00	99.99%	99.30%	99.34%	99.13%
Short term freezing: Rosenquist *et al.* (2006)	1.44	48.93%	30.51%	34.18%	32.97%
Short term freezing: Sandberg *et al.* (2005)	0.99	33.64%	20.97%	23.51%	22.69%
Short term freezing: Georgsson *et al.* (2006)	0.91	30.92%	19.28%	21.61%	20.86%
Long term freezing: Sandberg *et al.* (2005)	2.18	74.08%	46.16%	51.52%	49.66%
Long term freezing: Georgsson *et al.* (2006)	1.77	60.15%	37.49%	41.96%	40.46%
Lactic acid: Bolder (2007)	0.47	15.80%	9.85%	11.05%	10.66%
Hot water: Corry *et al.* (2006)	1.25	42.48%	26.48%	29.68%	28.64%
Acidified sodium chlorite: Kemp (2001)	1.75	59.47%	37.07%	41.49%	40.01%
Acidified sodium chlorite: Bashor *et al.* (2004)	1.26	42.81%	26.69%	29.92%	28.86%
Trisodium phosphate: Bashor *et al.* (2004)	1.03	35.00%	21.82%	24.46%	23.61%

(a): Reductions are same for indoor and outdoor flocks

Table 16: Effect of decontamination treatments in slaughter-house on the relative reduction in human cases in the four study countries, using the Beta-Poisson DR model

Treatment	Effect (log_{10} red)	C1	C2	C3	C4
Irradiation/cooking	6.00	99.99%	99.43%	99.46%	99.29%
Short term freezing: Rosenquist *et al.* (2006)	1.44	56.54%	24.88%	31.27%	29.21%
Short term freezing: Sandberg *et al.* (2005)	0.99	38.11%	15.92%	20.36%	19.00%
Short term freezing: Georgsson *et al.* (2006)	0.91	34.87%	14.44%	18.52%	17.28%
Long term freezing: Sandberg *et al.* (2005)	2.18	81.34%	41.85%	50.41%	47.37%
Long term freezing: Georgsson *et al.* (2006)	1.77	69.09%	32.13%	39.72%	37.17%
Lactic acid: Bolder (2007)	0.47	16.84%	6.86%	8.89%	8.30%
Hot water: Corry *et al.* (2006)	1.25	48.83%	20.96%	26.56%	24.79%
Acidified sodium chlorite: Kemp (2001)	1.75	68.42%	31.68%	39.20%	36.68%
Acidified sodium chlorite: Bashor *et al.* (2004)	1.26	49.29%	21.16%	26.80%	25.02%
Trisodium phosphate: Bashor *et al.* (2004)	1.03	39.57%	16.67%	21.29%	19.87%

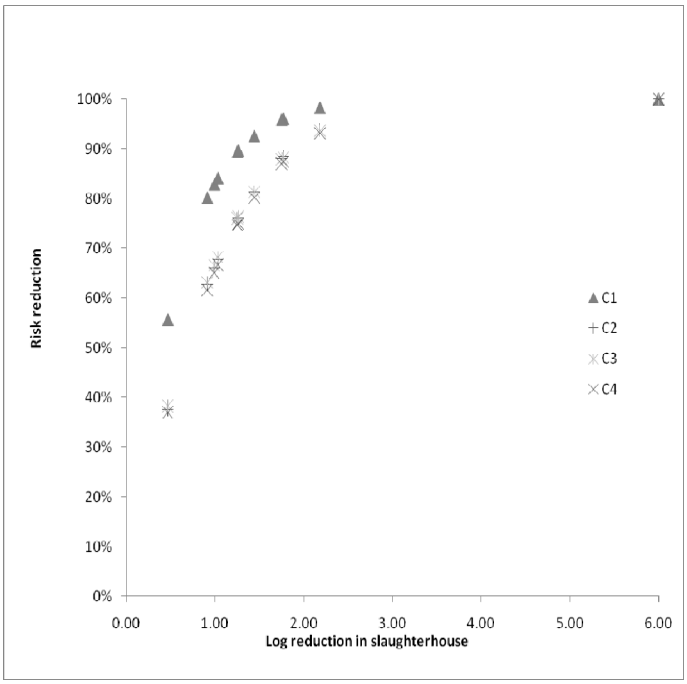

Figure 5: Effect of reductions of $\log_{10}$ *Campylobacter* counts on carcasses in the slaughter-house on the relative reduction in human cases using the Classic + DR model

3.8. Scheduled slaughter (testing pre-slaughter with decontamination of positive flocks)

3.8.1. Implementation

To model scheduled slaughter, the relative reduction in human cases from a selection of the interventions in Tables 12-14 was used and modified by the sensitivity of the test applied to the flocks as follows: Assuming perfect specificity of the tests (PCR and culture), the resulting risk reduction from applying the intervention to positive flocks only would be the product of the risk reduction found in Tables 12-14 and the sensitivity of the test used to identify positive flocks. The sensitivity estimates for the combination of sampling and testing are 75% using PCR (with sampling at least four days prior to slaughter, see Hofshagen and Opheim, 2008) and 48% for culture (Hofshagen and Kruse, 2005). Using data from the EU baseline survey, the percentage of flocks to which the intervention would be applied could similarly be calculated by multiplying the baseline prevalence estimate times the test sensitivity.

3.8.2. Results

The relative reduction in human cases by a selected number of interventions using scheduled slaughter are listed in Tables 15-20 for the four study countries. The reductions obtained by applying the interventions to all the flocks (i.e. without scheduled slaughter, as in Tables 12-14) have been included for ease of comparison. The impact on public health risk is dependent on a combination of the effectiveness of the intervention (as per simulation 3.7) and the sensitivity of the test. The proportion of flocks to which the intervention would be applied depends on the prevalence and the sensitivity of the test. The results follow broadly those found in simulation 3.7, although modulated by the sensitivity of the test.

Table 17: Risk reduction obtained when selected interventions are applied to positive flocks (scheduled slaughter), using PCR as detection test (assumed sensitivity=75%) using the Classic + DR model

		C1	C2	C3	C4
Prevalence		3.28%	30.27%	19.19%	75.81%
%Flocks to which intervention is applied with scheduled slaughter		22.70%	2.46%	14.39%	56.86%
Irradiation/heat treatment	Risk reduction if applied to all flocks	100.00%	100.00%	100.00%	100.00%
	Risk reduction with scheduled slaughter	75.00%	75.00%	75.00%	75.00%
Short term freezing: Sandberg *et al.* (2005)	Risk reduction if applied to all flocks	82.90%	66.14%	66.63%	65.10%
	Risk reduction with scheduled slaughter	62.18%	49.61%	49.97%	48.83%
Long term freezing: Sandberg *et al.* (2005)	Risk reduction if applied to all flocks	98.30%	93.98%	93.69%	93.16%
	Risk reduction with scheduled slaughter	73.73%	70.49%	70.27%	69.87%
Lactic acid: Bolder (2007)	Risk reduction if applied to all flocks	55.72%	37.56%	38.36%	37.01%
	Risk reduction with scheduled slaughter	41.79%	28.17%	28.77%	27.76%

Table 18: Risk reduction obtained when selected interventions are applied to positive flocks (scheduled slaughter), using culture as detection test (assumed sensitivity=48%) using the Classic + DR model

		C1	C2	C3	C4
Prevalence		3.28%	30.27%	19.19%	75.81%
%Flocks to which intervention is applied with scheduled slaughter		14.53%	1.58%	9.21%	36.39%
Irradiation/heat treatment	Risk reduction if applied to all flocks	100.00%	100.00%	100.00%	100.00%
	Risk reduction with scheduled slaughter	48.00%	48.00%	48.00%	48.00%
Short term freezing: Sandberg *et al.* (2005)	Risk reduction if applied to all flocks	82.90%	66.14%	66.63%	65.10%
	Risk reduction with scheduled slaughter	39.79%	31.75%	31.98%	31.25%
Long term freezing: Sandberg *et al.* (2005)	Risk reduction if applied to all flocks	98.30%	93.98%	93.69%	93.16%
	Risk reduction with scheduled slaughter	47.18%	45.11%	44.97%	44.72%
Lactic acid: Bolder (2007)	Risk reduction if applied to all flocks	55.72%	37.56%	38.36%	37.01%
	Risk reduction with scheduled slaughter	26.75%	18.03%	18.41%	17.76%

Table 19: Risk reduction obtained when selected interventions are applied to positive flocks (scheduled slaughter), using PCR as detection test (assumed sensitivity=75%) using the simple exponential DR model

		C1	C2	C3	C4
	Prevalence	3.28%	30.27%	19.19%	75.81%
%Flocks to which intervention is applied with scheduled slaughter		22.70%	2.46%	14.39%	56.86%
Irradiation/heat treatment	Risk reduction if applied to all flocks	99.99%	99.30%	99.34%	99.13%
	Risk reduction with scheduled slaughter	74.99%	74.48%	74.51%	74.35%
Short term freezing: Sandberg *et al.* (2005)	Risk reduction if applied to all flocks	33.64%	20.97%	23.51%	22.69%
	Risk reduction with scheduled slaughter	25.23%	15.73%	17.63%	17.02%
Long term freezing: Sandberg *et al.* (2005)	Risk reduction if applied to all flocks	74.08%	46.16%	51.52%	49.66%
	Risk reduction with scheduled slaughter	55.56%	34.62%	38.64%	37.25%
Lactic acid: Bolder (2007)	Risk reduction if applied to all flocks	15.80%	9.85%	11.05%	10.66%
	Risk reduction with scheduled slaughter	11.85%	7.39%	8.29%	8.00%

Table 20: Risk reduction obtained when selected interventions are applied to positive flocks (scheduled slaughter), using culture as detection test (assumed sensitivity=48%) using the simple exponential DR model

		C1	C2	C3	C4
	Prevalence	3.28%	30.27%	19.19%	75.81%
%Flocks to which intervention is applied with scheduled slaughter		14.53%	1.58%	9.21%	36.39%
Irradiation/heat treatment	Risk reduction if applied to all flocks	99.99%	99.30%	99.34%	99.13%
	Risk reduction with scheduled slaughter	48.00%	47.66%	47.68%	47.58%
Short term freezing: Sandberg *et al.* (2005)	Risk reduction if applied to all flocks	33.64%	20.97%	23.51%	22.69%
	Risk reduction with scheduled slaughter	16.15%	10.07%	11.28%	10.89%
Long term freezing: Sandberg *et al.* (2005)	Risk reduction if applied to all flocks	74.08%	46.16%	51.52%	49.66%
	Risk reduction with scheduled slaughter	35.56%	22.16%	24.73%	23.84%
Lactic acid: Bolder (2007)	Risk reduction if applied to all flocks	15.80%	9.85%	11.05%	10.66%
	Risk reduction with scheduled slaughter	7.58%	4.73%	5.30%	5.12%

Table 21: Risk reduction obtained when selected interventions are applied to positive flocks (scheduled slaughter), using PCR as detection test (assumed sensitivity=75%) using the Beta-Poisson DR model

		C1	C2	C3	C4
	Prevalence	3.28%	30.27%	19.19%	75.81%
%Flocks to which intervention is applied with scheduled slaughter		22.70%	2.46%	14.39%	56.86%
Irradiation/heat treatment	Risk reduction if applied to all flocks	99.99%	99.43%	99.46%	99.29%
	Risk reduction with scheduled slaughter	74.99%	74.57%	74.60%	74.47%
Short term freezing: Sandberg *et al.* (2005)	Risk reduction if applied to all flocks	38.11%	15.92%	20.36%	19.00%
	Risk reduction with scheduled slaughter	28.58%	11.94%	15.27%	14.25%
Long term freezing: Sandberg *et al.* (2005)	Risk reduction if applied to all flocks	81.34%	41.85%	50.41%	47.37%
	Risk reduction with scheduled slaughter	61.01%	31.39%	37.81%	35.53%
Lactic acid: Bolder (2007)	Risk reduction if applied to all flocks	16.84%	6.86%	8.89%	8.30%
	Risk reduction with scheduled slaughter	12.63%	5.15%	6.67%	6.23%

Table 22: Risk reduction obtained when selected interventions are applied to positive flocks (scheduled slaughter), using culture as detection test (assumed sensitivity=48%) using the Beta-Poisson DR model

		C1	C2	C3	C4
	Prevalence	3.28%	30.27%	19.19%	75.81%
%Flocks to which intervention is applied with scheduled slaughter		14.53%	1.58%	9.21%	36.39%
Irradiation/heat treatment	Risk reduction if applied to all flocks	99.99%	99.43%	99.46%	99.29%
	Risk reduction with scheduled slaughter	48.00%	47.73%	47.74%	47.66%
Short term freezing: Sandberg *et al.* (2005)	Risk reduction if applied to all flocks	38.11%	15.92%	20.36%	19.00%
	Risk reduction with scheduled slaughter	18.29%	7.64%	9.77%	9.12%
Long term freezing: Sandberg *et al.* (2005)	Risk reduction if applied to all flocks	81.34%	41.85%	50.41%	47.37%
	Risk reduction with scheduled slaughter	39.04%	20.09%	24.20%	22.74%
Lactic acid: Bolder (2007)	Risk reduction if applied to all flocks	16.84%	6.86%	8.89%	8.30%
	Risk reduction with scheduled slaughter	8.08%	3.29%	4.27%	3.98%

D. THE IMPACT OF THE IMPLEMENTATION OF MICROBIOLOGICAL CRITERIA

Method

Here we use a risk assessment approach to explore the potential impact on public health of setting microbiological criteria (MC) for *Campylobacter* on chicken meat.

The criterion is set at the point in the chain (immediately after chilling) where skin samples were taken in the EU baseline survey (EFSA, 2010). We assume the methods applied for sampling and analysis when testing for compliance with the MC are the same as in the EU baseline survey.

We assume a microbiological criterion will be defined in terms of

- sample size n,

- critical limit m (maximum concentration per g)

- c, the critical number of samples that may yield a value larger than m.

- the analytical method for detection and enumeration has the same performance characteristics as the one used in the baseline study

We do not state whether the criterion will be a Food Safety Criterion (FSC) or a Process hygiene Criterion (PHC). However, we assess the maximum effect that can be achieved by the MC setting by assessing the *minimum relative residual risk* (MRRR) in the population. This assumes that ***all*** batches of meat that do not comply with the MC are diverted away from the fresh meat market, and receive a treatment that destroys all *Campylobacter*. And it assumes that ***all*** batches are tested, and therefore this is a *maximum* achievable risk reduction, providing a *minimum* residual risk.This is the assessed human incidence due to *Campylobacter* on broiler meat from batches that comply with the microbiological criterion divided by the assessed human incidence before implementation of the MC (which is in the baseline).

Model for *Campylobacter* Microbiological Criteria (CAMC)

In order to estimate the effect of setting MC, another model was developed.

The baseline data on carcasses consists of enumeration data and detection after enrichment data for the same samples, taken from one batch of broiler meat.

We assume that all batches where enumeration data were obtained and all batches where *Campylobacter* was detected on the carcasses were truly positive. Those that tested negative after enrichment and did not yield plate counts are considered truly negative. The prevalence is the fraction "not truly negative" according to this definition, and is also the prevalence mentioned in Table 12 of the EU baseline surevey report: Part A {EFSA, 2010 #11453}.

We fit a normal distribution through the enumeration data, and assume the positives that could not be enumerated have a concentration lower than the detection limit (10 CFU/g) and treat them as left censored. We used a Markov Chain Monte Carlo procedure programmed in SAS (Cary, NC, USA) to estimate the mean and standard deviation of the $\log_{10}$ colony-forming units of *Campylobacter* per g. The distribution was assumed log-normal. We use proper but diffuse priors on both mean and standard deviation, respectively Normal with mean equal to zero and standard deviation equal to 1000 and Gamma distribution with shape and scale equal to 0.001. From the posterior distribution of *Campylobacter* concentration mean and standard deviation we consider their median for the risk assessment model.

We do not use the data from Cyprus and Estonia, because too few samples were enumerated. We have no data from Greece, Luxemburg or Iceland.

Table 23: Contamination status of broiler meat in different MSs (plus Norway and Switzerland), expressed in terms of prevalence, mean (μ_{est}) and standard deviation (sd_{est}) on log scale.

MS	μ_{est}	Sd_{est}	prevalence
AT	2.18	1.38	80.6%
BE	2.72	0.93	52.1%
BG	2.87	1.04	45.0%
CZ	2.35	1.58	69.9%
DK	2.11	1.28	31.1%
FI	0.32	1.59	5.7%
FR	2.13	1.01	87.7%
DE	1.87	1.66	62.0%
HU	2.19	1.19	56.1%
IE	2.72	1.01	98.0%
IT	2.72	1.01	52.2%
LV	2.02	0.78	33.6%
LT	1.96	0.93	46.0%
MT	1.96	0.93	94.8%
PL	2.87	0.97	80.9%
PT	2.50	1.05	74.1%
RO	2.50	1.05	63.6%
SK	2.68	1.06	74.6%
SI	1.72	0.78	80.6%
ES	2.86	1.18	100.0%
SE	1.48	1.01	13.4%
NL	2.34	1.14	37.8%
UK	2.27	1.33	87.3%
NO	0.95	0.51	5.1%
CH	42.10	1.41	70.6%

In the EU baseline survey one skin sample was taken per batch. This means that the extent to which the variance reflects within-batch and between-batch variance is unknown. We assume that a fraction α of the variance is attributable to within-batch variance, such that

$\alpha\ var_{est} = var_{within\ batch}$.

We assume that the within-batch variance is fixed for all batches, and the mean $\log_{10}$ count per batch μ is assumed to have a normal distribution, with mean μ_{est} and variance $(1-\alpha)\ var_{est}$

We assume that α< 50% and perform a sensitivity analysis for different values.

For each batch we

1) calculate the probability of (not) complying with the MC, taking *n* samples, evaluating how many of them give a value > *c*, given a value *m*. We assume a perfect test, with sensitivity and specificity of 100%.

Given a normal distribution of log concentrations on the skin

$C \sim N(\mu, sd_{within\ batch})$

the probability P(C>*m*) is calculated from the normal distribution cumulative density function.

Next, the probability of getting more than *c* out of *n* samples not complying with the criterion P(X>c) follows from the probability function of the Binomial (N,p) distribution with N = *n* and p = P(C>*m*).

Hence, the probability obtained is a function of the distribution of concentrations found in the EU baseline survey and the values of *n*, *c* and *m*. It can be calculated by integration. This is approximated in an Excel spreadsheet dividing up the normal distributions into 61 bins.
This probability is the expected percentage of batches that is affected by the criterion, and in the most extreme scenario the percentage of batches diverted away from fresh meat production. In a less extreme scenario it is the percentage of tested batches that indicate that action should be taken. We refer to this as the percentage of batches not complying with the criterion (BNMC).

2) Assess the risk, using the classic + "dose response" model (Nauta and Christensen, 2010) with the unknown transition parameter indicating the log difference between CFU per gram of skin and CFU per gram of meat set at $\tau = 1$. A sensitivity analysis for different values of τ is done. With this model, the probability of illness is a function of the distribution of skin concentrations, C, in a batch. The probability of illness per batch (where the distribution of C is known) can be calculated by integration.

The risk can be assessed for all batches (from a MS or the whole of the EU weighted for the amount of meat produced in 2008) and only for those batches complyin with the criterion. Dividing these risks yields the minimum relative residual risk (MRRR). For each country, the risk for contaminated batches is multiplied by the batch prevalence to arrive at a population risk estimate.

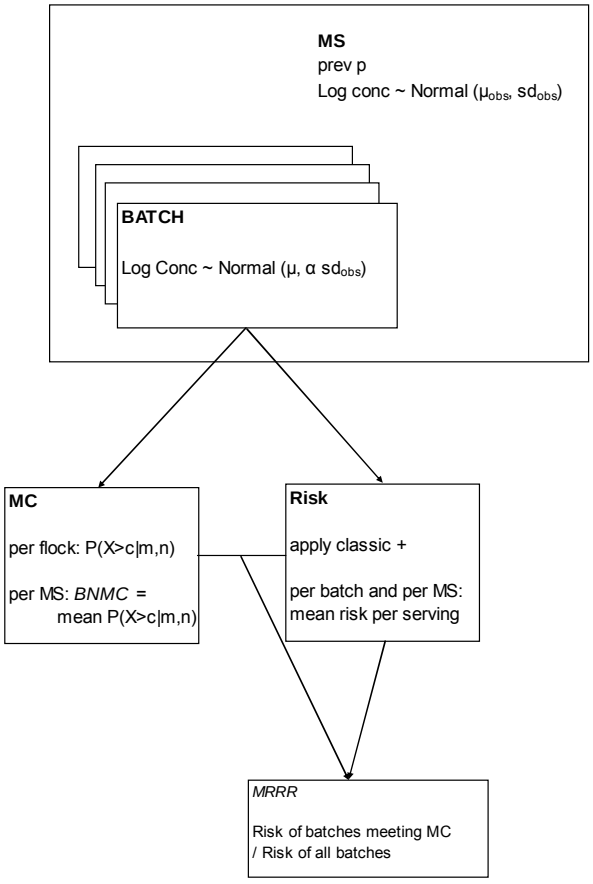

Figure 6: Outline of the model used to estimate the effect of microbiological criteria

Results

First, consider a default scenario, with n=5, c= 1 and m = 1000, $\alpha = 30\%$, $\tau = 1$.

The minimum residual risks (MRRs) and percentages of batches not complying with the criteria (BNMC) can now be calculated for each MS, and for the whole of Europe, by weighing the individual results of the MSs by their contribution to the total European production.

Figures 2 and 3 show the results of this scenario. Current risks vary largely between MSs, the minimum residual risks vary to a smaller extent. The percentage of non-compliant batches varies widely as well. The MC actually seems to yield a good prediction of the risk in an MS: the more batches found to be positive, the higher the current risk. Figure 3 shows the relationship between BNMC and MRRR in different EU MSs. In general, MRRR is somewhat lower for a criterion with m=1000, n=3, c=0 than for m=1000, n=5 and c=1; while BNMC is higher.This indicates that an MC with just 3 samples may perform as well or even slightly better than an MC with 5 samples.

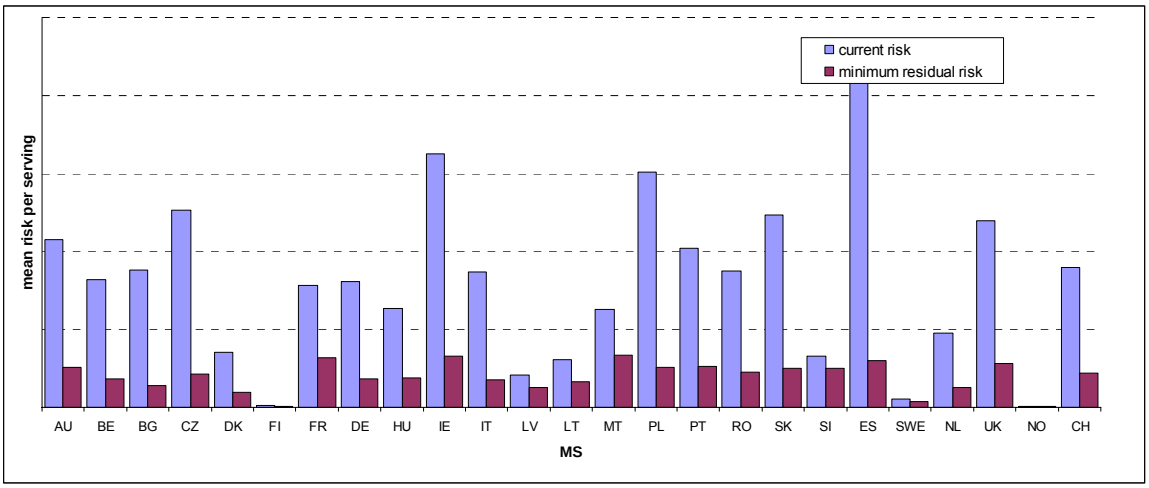

Figure 7: Comparison of the assessed mean risks per serving for broiler meat produced in the different EU MSs plus Norway and Switzerland as follows from the EU baseline survey data and the classic + DR model (current risks) and the minimum residual risks with implementation of the MC with m= 1000, n=5, c=1. Values of the mean risk are on a linear scale, but they are not given as they have to be read relative to each other. The MRRR value used elsewhere equals the value of the minimum residual risk divided by the current risk.

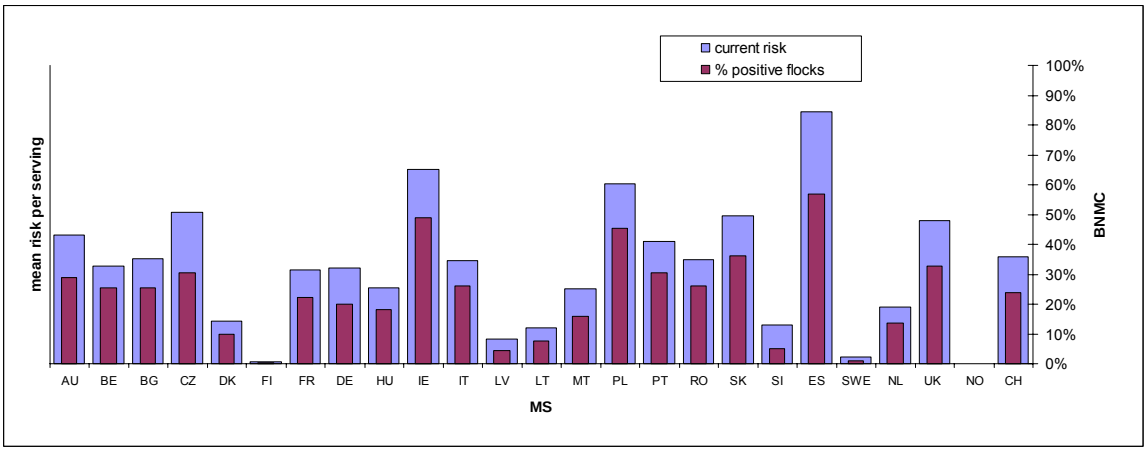

Figure 8: The assessed mean risks per serving for the broiler meat produced in the different EU MSs as follows from the EU baseline survey data and the classic + DR model (current risks) and the percentage of batches not complying with the MC (BNMC) with implementation of the MC with m= 1000, n=5, c=1. Note that the latter should be read from the secondary y axis.

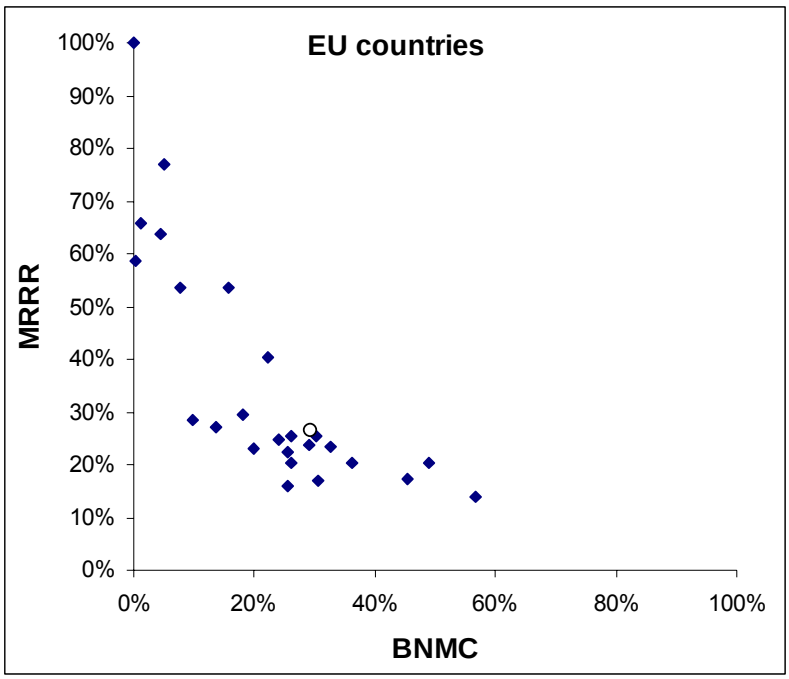

Figure 9: A scatter plot of percentage of batches not complying with the criterion (BNMC) and the minimum relative residual risk (MRRR) after implementation of the MC with m= 1000, n=5, c=1 for the different MSs. The EU mean is given by the small circle. Note that, ideally, both the MRRR and BNMC are low. The variability between the EU MSs is large. If the MRRR is high and the BNMC is low, this usually means the current status in an MS is better.

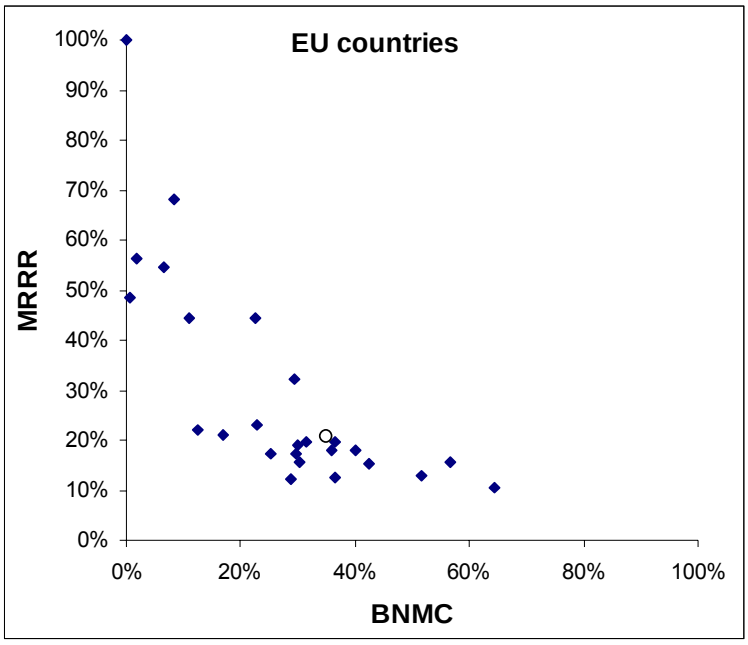

Figure 10: A scatter plot of percentage of batches not complying with the criterion (BNMC) and the minimum relative residual risk (MRRR) after implementation of the MC with m=1000, n=3, c=0 for the different MSs. The EU mean is given by the small circle. Note that, ideally, both the MRRR and BNMC are low. The variability between the EU MSs is large. If the MRRR is high and the BNMC is low, this usually means the current status in an MS is better.

Interestingly, the BNMC is a very good indicator of the current risk, a lot better than the prevalence. This is illustrated in Figure 6.

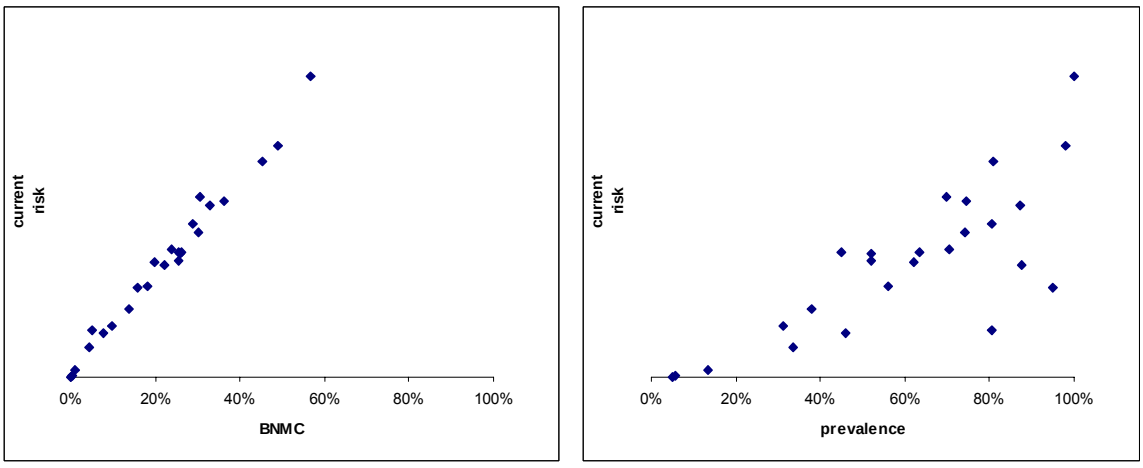

Figure 11: The relation of the current risk for the MCs and with the prevalence, for the baseline sampling scheme m=1000, n=5, c=1.

Other MC

Next, we explore the impact of other sampling schemes for the MC with m={100, 500, 1000, 5000, 10000} and {n,c} combinations {{1,0},{3,0},{3,1},{5,0},{5,1}, {5,2},{10,0},{10,1},{10,2}}. As a default we look at the EU mean for the results.

First, it can be seen that the BNMC decreases with increasing m (see Figure 7), which is not particularly surprising. This is however an important output of the analysis, because the % positive

batches are those where action should be taken, which means they incur costs no matter how the MC is implemented (as FSC or PHC, for all batches or a (small) percentage of sampled batches only).

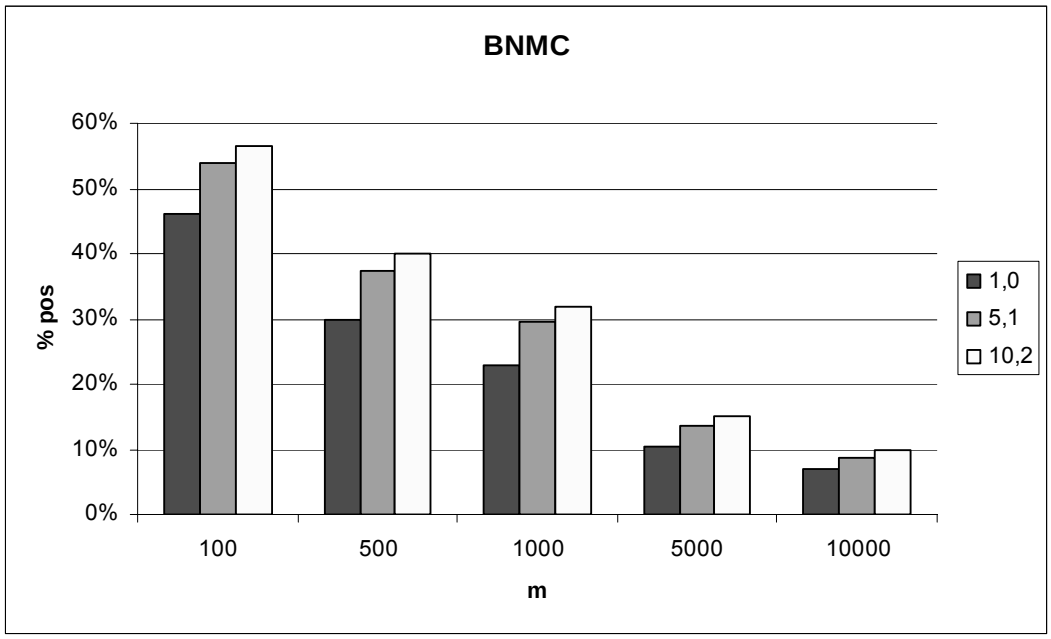

Figure 12: Batches not complying with the microbiological criterion (BNMC) for different values of the treshold m, for three combinations of {n,c} = {1,0}, {5,1} and {10,2}. Results are the weighted EU means and will be different between MSs.

An important result is the relation between BNMC and MRRR. The BNMC can be regarded as the cost (the more batches are positive, the more action has to be taken) and should be as low as possible. The minimum relative residual risk (MRRR) should also be as low as possible. Different sampling schemes yield different BNMC and MRRR, and the risk manager can in principle decide on the optimum sampling scheme based on the expected results. Costs for sampling will be higher if more samples are to be taken, so preferably n is low. The results show that a higher *n* does increase the efficiency of the test (i.e. low MRRR and low BNMC), but only very little (see Figures 8 and 9).

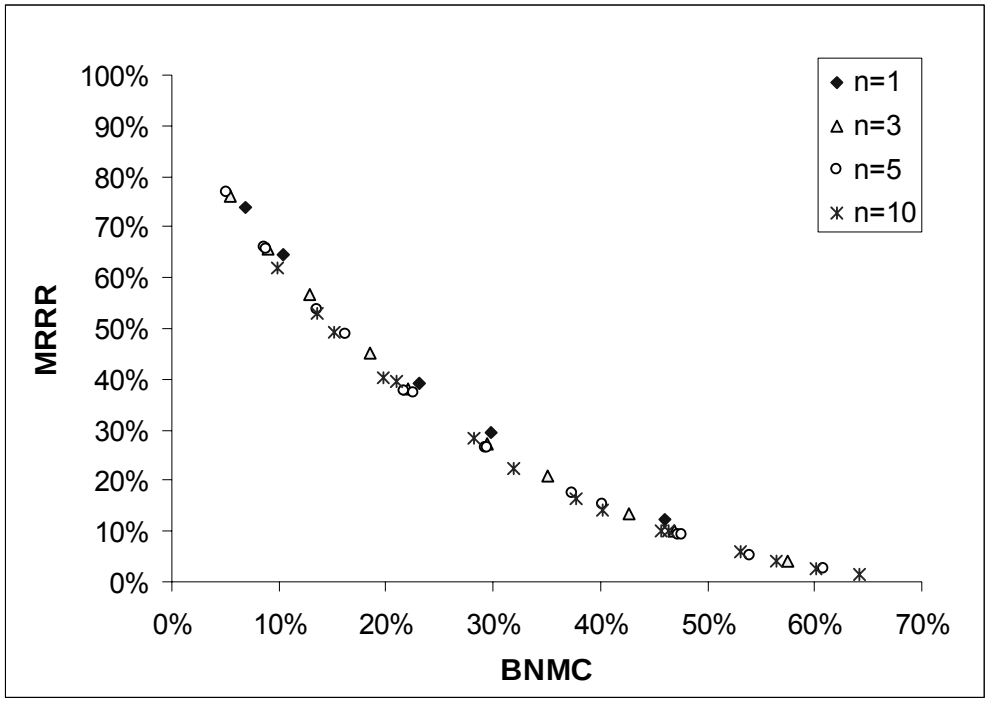

Figure 13: Batches not complying wth the microbiological criterion (BNMC) versus minimum relative residual risk (MRRR) for the weighted EU mean. When many batches do not meet the criterion, the residual risk once they are diverted away from fresh meat production is small; the residual risk is higher if fewer batches are diverted away. Increasing the sample size does not help very much. With higher m and higher c fewer batches are positive and the MRRR is higher.

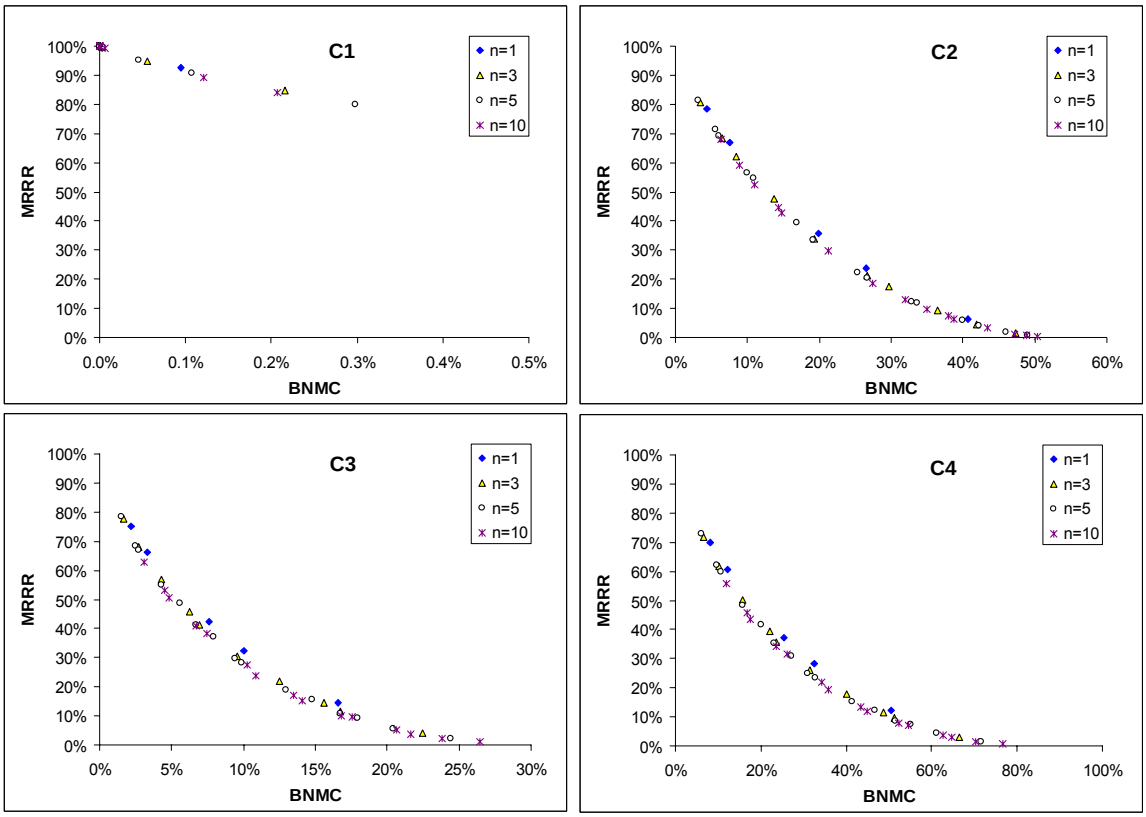

Figure 14: Batches not complying wth the microbiological criterion (BNMC) versus minimum relative residual risk (MRRR) for the study countries C2, C3, C1 and C4.The relations between BNMC and MRRR differ between countries. . Note the different scales on the x-axis.

Table 24 suggests that even when applying a high value for m, considerable risk reduction can be expected. For example, with m=10,000, n=5 and c=1, the residual risk would be 661% (risk reduction 34%), while 9% of batches would be rejected on average. 19/25 countries would reject less than 10% of the batches, whereas 1/25 countries would reject more than 20% with a maximum of 22% batch rejection. Risk reduction would be less than 50% in 23 countries and 50-75% in another two.

Table 24: Evaluation of different values for m, n and c for the EU as a whole

m	n	c	Residual risk	batch rejection
100	1	0	12.2%	46.1%
	3	0	4.1%	57.5%
		1	9.9%	46.9%
	5	0	2.7%	60.9%
		1	5.3%	54.0%
		2	9.2%	47.2%
	10	0	1.6%	64.2%
		1	2.7%	60.0%
		2	4.0%	56.4%
500	1	0	29.3%	29.8%
	3	0	13.5%	42.7%
		1	27.3%	29.4%
	5	0	9.4%	47.7%
		1	17.4%	37.4%
		2	26.5%	29.2%
	10	0	5.9%	53.1%
		1	10.0%	45.7%
		2	14.2%	40.1%
1000	1	0	39.3%	23.0%
	3	0	20.8%	35.2%
		1	38.1%	22.0%
	5	0	15.3%	40.3%
		1	26.4%	29.4%
		2	37.7%	21.7%
	10	0	10.1%	46.3%
		1	16.5%	37.8%
		2	22.4%	32.0%
5000	1	0	64.4%	10.4%
	3	0	45.0%	18.5%
		1	65.7%	9.0%
	5	0	37.2%	22.6%
		1	53.7%	13.6%
		2	66.2%	8.6%
	10	0	28.3%	28.2%
		1	40.4%	19.8%
		2	49.4%	15.1%
10000	1	0	74.0%	6.8%
	3	0	56.8%	12.9%
		1	76.1%	5.5%
	5	0	49.0%	16.2%
		1	65.7%	8.8%
		2	76.8%	5.1%
	10	0	39.5%	20.9%
		1	53.0%	13.6%
		2	62.0%	9.84%

Decreasing the value for m would result in progressive risk reduction and increased batch rejection. With n=5 and c=1, the following results were obtained.

Table 25: Relative risk reduction and batch rejection for different values of m

M	10,000	5,000	1,000	500	100
Minimum relative residual risk	66%	54%	26%	17%	5%
Batch rejection	9%	14%	29%	37%	54%.

According to the model results, MCs that would result in at least 50% risk reduction for the EU as a whole are: any MC with

- m=100, 500, 1000;
- m=5,000 with n=3, c=0; n=5, c=0; or n=10, c=0; and n=10, c=1;
- m=10,000, n=10, c=0.

A risk reduction of at least 90% can only be obtained by MC with

- m=100, n=3, c=0; n=5, c=0, 1 or 2 and n=10, c=0,1 or 2;
- m=500, n=10, c=0 and c=1.

Sensitivity analysis

Results with different values for α and τ

We assumed values for α and τ. The sensitivity of the output for these parameters is studied.

It shows that the impact of α is not very large. The BNMC and MRRR values for the baseline sampling scheme m=1000, n=5, c=1 are given for the EU mean and four different MS in Table 4.

Table 26: The BNMC and MRRR values for the baseline sampling scheme m=1000, n=5, c=1 are given for the EU mean and four study countries.

alpha	EU		C2		C3		C4	
	MRRR	BNMC	MRRR	BNMC	MRRR	BNMC	MRRR	BNMC
10%	22.9%	26.7%	21.0%	23.0%	23.2%	9.0%	19.0%	29.7%
30%	26.4%	29.4%	22.5%	25.4%	28.3%	9.9%	23.4%	32.7%
50%	31.5%	31.4%	25.2%	27.3%	35.6%	10.4%	29.6%	34.9%

It can be seen that both MRRR and BNMC increase with increasing within batch variability, which means that in general a microbiological criterion will be less efficient when the within batch variability increases. This is particularly relevant if batches constituted a mixture of meat from different flocks.

τ is the parameter in the classic + model that defines the difference between the log concentration per g of meat and the log concentration per g of skin as measured in the baseline study. It has no impact on the BNMC, but only on the MRRR (see table below).

The MRRR is higher with lower τ. The range found may be used as an indicator of the uncertainty about the estimate.

Table 27: Impact of tau on the MRRR

tau	EU	C2	C3	C4
0	33.7%	29.1%	37.0%	31.4%
1	26.4%	22.5%	28.3%	23.4%
2	19.1%	15.7%	19.7%	15.8%

ABBREVIATIONS

ALOP	appropriate level of protection
ASC	acidified sodium chlorite
BFP	between-flock prevalence
BNMC	batches not complying wity microbiological criteria
c	number of sample units on which m is exceeded
CAMC	Model for CAmpylobacter Microbiological Criteria
CAMO	commissioned model CAmpylobacter MOments
CamPrev	model for Campylobacter Prevalence targets
CE	competitive exclusion
DALY	disability-adjusted life year
DR	dose-response
EO	electrolyzed oxidizing
FSC	food safety criterion
FSO	food safety objective
GHP	good hygienic practices
GMP	good management practices
HACCP	hazard analysis and critical control points
HPP	hydrostatic pressure processing
IOBW	inside-outside bird washer
m	limit to microbiological counts in CFU/g of skin sample
MC	microbiological criteria
MRRR	miminal relative residual risk
MS	Member States
n	number of units comprising the sample
PA	peracetic or peroxyacetic acid
PC	performance criteria
PCR	polymerase chain reaction
PFGE	pulse field gel electrophoresis
PHC	process hygiene criterion
PO	performance objective
PrC	process criteria
QMRA	quantitative microbiological risk assessment
SD	standard deviation
SMI	Swedish Institute for Communicable Disease Control (Smittskyddsinstitutet)
SmiNet	Swedish infectious disease surveillance system
TSP	trisodium phosphate