Post-consultation draft for final Committee sign-off

ACM/1295b

24/06/2019

Advisory Committe on the Microbiological Safety of Food

Ad Hoc Group on *Campylobacter*

Third Report on *Campylobacter*

Advises the Food Standards Agency on the Microbiological Safety of Food

Contents

Summary

1. This is the Third Report of the Advisory Committee on the Microbiological Safety of Food (ACMSF) dealing with *Campylobacter*. We have returned to review this topic over a decade after our Second Report on the subject because of the continued dominance of *Campylobacter* as the leading bacterial cause of foodborne disease in the UK.

2. Our terms of reference were to assess the actions that have taken place since the publication of the Second *Campylobacter* Report and to make proposals to advise the Food Standards Agency in evolving its strategy for reducing the incidence and risk of foodborne *Campylobacter* infection in humans.

3. We established an Ad Hoc group in 2016 to carry out this review. The membership comprised members of the ACMSF (Prof Sarah O'Brien (Chair), Mr Alec Kyriakides, Prof Peter McClure, Prof David McDowell, Mr David Nuttall and Dr Dan Tucker) and co-opted experts (Prof Tom Humphrey (University of Swansea), Mrs Joy Dobbs (Social Science Research Committee), Prof Norval Strachan (University of Aberdeen), Prof Noel McCarthy (University of Warwick), Prof Martin Maiden (University of Oxford) and Mrs Ann Williams (Consumer rep)). We were ably assisted by the Secretariat (Dr Manisha Upadhyay (Scientific Secretary), Mr Adam Hardgrave (FSA policy representative), Mr Adekunle Adeoye and Ms Sarah Butler).

4. This Report represents the output of the *Ad Hoc* Group members' deliberations and updates the scientific evidence since the publication of our last report. The chapter contents are described briefly below. Although our focus is on the UK situation, we have drawn on the international scientific literature where appropriate.

5. In Chapter 1 (*Campylobacter* biology and tools for detection) we describe advances in understanding of the biology of *Campylobacter* spp. The chapter includes an update on the general characteristics of these important pathogens, considers how they respond to different environmental stresses and describes tools for their detection. We conclude that increased understanding of mechanisms of stress response and biology of *Campylobacter* has revealed a number of alternative mechanisms that allow the bacteria to survive under stress conditions. However, this has yet to lead to

development of new strategies for improved control. We also comment on evidence that some strains can become hyper aero-tolerant, surviving much longer than aero-sensitive strains and there is some suggestion that these may be more virulent than aero-sensitive strains. This suggests that *Campylobacter* spp. might not be as fragile as previously thought.

6. In Chapter 2 (*Campylobacter* genetics and genomics) we summarise the significant advances that have been made in the genetic analysis of pathogens and the application of these techniques to improve our understanding of *Campylobacter* genetics and genomics. We conclude that nucleotide sequence analyses have enabled substantial advances to be made in the understanding of the biology of *C. jejuni* and *C. coli*. We note that the development of high-resolution near-patient characterisation, preferably from complex clinical specimens, remains a major goal.

7. In Chapter 3 (Epidemiology of *Campylobacter* infection in humans) we provide an update on the epidemiology of *Campylobacter* infection in humans including disease burden, seasonality, and contaminated food vehicles implicated in outbreaks and risk factor studies. We conclude that *Campylobacter* remains the most common confirmed bacterial cause of acute gastroenteritis in the UK and that routine surveillance remains key to understanding trends. We note that contaminated poultry remains the greatest risk to humans, but avoidable infections are also re-emerging in the UK associated with consuming raw (unpasteurised) milk. We comment that it is not entirely clear that interventions in the food chain have yet led to a sustained reduction in human disease.

8. In Chapter 4 (Source attribution of human campylobacteriosis) we review the use of microbiological characterisation, and specifically analyses of *Campylobacter* genetic information to identify sources of human infection, which draws on the methods described in Chapter 2 and complements the epidemiological evidence presented in Chapter 3. We conclude that genetic sequencing technologies have removed issues such as reproducibility and non-typeable strains and all *Campylobacter* isolates can now be reliably characterised in a way that is mainly only limited by the actual level of existing biological variation. We note that source attribution methods generate more accurate information to guide risk assessment and management.

9. In Chapter 5 (Risks in the food chain: Poultry) we focus on the risks in the food chain from poultry, now implicated, either directly or indirectly, in up to 80% of human *Campylobacter* infections. We conclude that, from a human health perspective, *Campylobacter*-contaminated chicken seems to pose several threats: high surface levels, a cross-contamination risk, and infection of edible tissues, including chicken liver and chicken muscle. We note that no single practical intervention has been shown to be capable of eliminating *Campylobacter* spp. or even reducing it to acceptable levels in the bird or during processing. However, levels can be reduced by a combination of farm and processing controls that include implementation of improved biosecurity measures on farm e.g. hygiene barriers in sheds, time-controlled depopulation and in the process e.g. optimisation of existing processing, application of thermal processing (hot or cold). We also note that a key factor in the initial success achieved by the industry in reducing the levels of *Campylobacter* spp. in UK chicken was a full supply chain approach and the importance of promoting an open, collaborative approach is recommended for this and other industry challenges.

10. In chapter 6 (Risks in the Food Chain: Measures to prevent *Campylobacter* contamination of chicken meat in Europe, New Zealand and the USA) we draw on evidence from other countries about measures to prevent contamination of chicken meat with *Campylobacter*. We describe experiences and lessons learned from Europe, New Zealand and the USA.

11. In Chapter 7 (Risks in the food chain: Red meat, raw milk and fresh produce) we focus on food-related risk factors other than poultry. We conclude that red meat presents a low risk for food-borne transmission of pathogenic *Campylobacter* spp. to consumers. Available evidence indicates that existing process controls, especially chilling of carcasses, provide an effective means for control of *Campylobacter* along red meat supply chains. We note an emerging risk from consumption of raw (unpasteurised) milk, but that pasteurised milk poses an extremely low risk of campylobacteriosis.

12. In Chapter 8 (People's attitudes and behaviours regarding risk (includes consumers, caterers, farmers and the food processing industry)) we concentrate on the knowledge, attitudes and behaviour relating to current risks posed by *Campylobacter* within the human food chain among people involved in the production

and consumption of foods likely to contain *Campylobacter*. That includes farmers and others working in either primary production or food industry processing, people working in the catering industry and, importantly, consumers.

13. In Chapter 9 (How new knowledge influences risk assessment) we use a risk governance framework to identify what activity has gone well and what has not gone well in terms of understanding and trying to reduce the levels of human campylobacteriosis in the UK.

14. Finally, in Chapter 10 (Conclusions and Recommendations), we summarise, for ease of reference, the conclusions we have drawn in this Report and the recommendations we have made.

Chapter 1: *Campylobacter* biology and tools and for detection

Introduction

1.1 Since publication of the previous ACMSF report, there have been several advances in understanding the biology of *Campylobacter* and the main purpose of this chapter is to provide an update on the general characteristics of these important pathogens, how they respond to different environmental stresses and tools for their detection.

Taxonomy

1.2 Currently, 35 species are recognised within the genus *Campylobacter* (1), with *Campylobacter jejuni* being responsible for the majority of human illnesses, but disease is also caused by *C. coli*, and to a lesser extent, *C. lari* and *C. upsaliensis.* This group is referred to as thermophilic *Campylobacter* species. These four *Campylobacter* are zoonotic pathogens naturally present in the gastro-intestinal tract of both domestic and wild animals. The evidence for and role of *Campylobacter* in disease in poultry is discussed later in Chapter 5.

General characteristics

1.3 *Campylobacter* are Gram-negative, micro-aerophilic, non-spore forming, small vibroid (spiral-shaped) cells that have rapid, darting, reciprocating motility. They reduce nitrate and nitrite (apart from *C. jejuni* subsp. *doylei* and *C. fennelliae*), are unable to oxidise or ferment carbohydrates and obtain energy from amino acids or tricarboxylic acid cycle intermediates. *Campylobacter jejuni* is generally considered to be more susceptible to environmental conditions compared to some other non-spore-forming, infectious, bacterial foodborne agents, such as salmonellae. *Campylobacter* is generally considered to be heat sensitive when compared to other infectious foodborne pathogenic bacteria, although a few studies report higher resistance in large pieces of poultry meat (see below). The organisms are part of the intestinal microbiota of a wide variety of wild and domestic animals, where optimal temperatures (37-43^{o}C) and microaerophilic environments favour their growth. The 42^{o}C body temperature of poultry is closest to the optimal growth temperature of thermophilic *Campylobacter*. The amino acids in the gut of poultry are a rich source of carbon for these organisms.

1.4 The virulence of the organisms, as suggested by the relatively low infectious dose of a few hundred cells in humans, and its widespread prevalence in animals are important features that explain why these organisms, considered to be relatively sensitive compared to other food borne pathogens, are a leading cause of gastroenteritis in man. *Campylobacter jejuni* tends to predominate in cattle, broiler chickens and turkeys while *C. coli* is more commonly found in pigs.

1.5 Isolates of *Campylobacter* are genetically diverse, compared to some other enteropathogens. This diversity may be partly explained by the natural competency of *Campylobacter* spp. to take up DNA. The high levels of multiple-strain colonisation and high frequency of incidence in mammals and birds mean there is substantial opportunity for exchange of genetic material. Genetic diversity, as evidenced by the different genotypes and phenotypes observed (through PFGE, RAPD, ribotyping, AFLP etc) is indicative of genomic plasticity – the order of genes on the chromosome is not conserved between isolates of the same species. Sub-types recognised by one phenotypic or genotypic method often do not correlate with other techniques. It is thought that the high frequency of intragenomic recombination events enhances the ability to survive and adapt to a range of adverse conditions (2, 3).

Growth and survival characteristics of *Campylobacter*

1.6 The gastrointestinal tract of poultry is a harsh environment and colonisation followed by persistence suggests that these organisms are capable of adaptive responses to different environments. Survival of *C. jejuni* outside the gut is believed to be relatively poor and the organism is sensitive to drying, freezing, and low pH (pH $\leq$ 4.7). *Campylobacter* spp. lack the presence of stationary phase sigma factor gene *rpoS,* which encodes for the global regulator sigma 38, and therefore lack many of the adaptive responses present in other bacteria. *Campylobacter* spp. also lack other factors such as the oxidative stress response factors SoxRS, the cold-shock response protein CspA, RpoH or Lrp, that are found in many other bacteria. In *Campylobacter*, the expression of survival and virulence genes is controlled by the *fliA*, *rpoN*, and *rpoD* genes, which encode for sigma factors 28, 54 and 70 respectively. In addition, *spoT* controls the stringent stress response in *C. jejuni*. Mutants that lack this gene demonstrate lower aerotolerance, rifampicin resistance, stationary phase survival, adherence ability, invasive capability and survival within intestinal cells (4). The main stresses that *Campylobacter* face in environments

outside their primary hosts include desiccation, oxidative stress and lower temperatures.

Osmotic stress

1.7 *Campylobacter* is not able to grow in NaCl concentrations greater than 2% or at an a_w of below 0.987. Below this a_w cells die quickly, particularly at higher temperatures. Kusumaningrum *et al* (5) did not detect any surviving *Campylobacter* cells on stainless steel surfaces that had been stored at room temperature for 4 h, starting with an inoculum as high as 10^7 cfu/100 cm^2. Modified atmospheres appear to have little effect on survival. This reduced tolerance to low a_w has been shown to have an impact on colonisation rates in broilers, where Line *et al* (6) reported that litter a_w at 0.5 reduced colonisation in chicks compared to that at a_w 0.795. A delay in *Campylobacter* colonisation was shown in birds raised under low a_w conditions, which increased with increasing time between removal of birds and placement of newly-hatched chicks. This study concluded that lag times between flocks of at least 1 week are unlikely to result in *Campylobacter* infection of subsequent flocks of birds from previously contaminated litter.

1.8 Much like *Listeria*, *Campylobacter* morphology changes under hyperosmotic stress, with cells elongating due to septum defects. Under low osmotic pressure, cells form a coccoid shape, correlated with viable but non-culturable (VBNC) cells that have been commonly reported for *Campylobacter* spp. under stress conditions. *Campylobacter* resists high osmotic pressure better at lower temperatures. This feature, though not unique to *Campylobacter*, is sometimes overlooked by researchers who use growing cells in stress conditions, potentially leading to misleading results indicating more rapid die-off than would otherwise occur under common environmental conditions. The mechanisms by which *Campylobacter* spp. are thought to survive high osmotic stress include amino acid uptake, controlled by genes regulated by ppGpp (guanosine triphosphate). A more detailed review of osmotic stress response in *Campylobacter* is provided by Burgess et al (7). A major role for *rpoN* has been reported for *Campylobacter* (8). Many other pathogenic bacteria can survive high osmotic pressure by increased production or accumulation of compatible solutes such as trehalose, glycine betaine or proline. However, *Campylobacter* do not possess these capabilities and this may explain why

alternative strategies are required even though these may be less effective than more commonly understood mechanisms in other bacteria.

1.9 *Campylobacter* is sensitive to desiccation (9) and the organisms can die on the surfaces of red meat (lamb, beef and pork) carcasses in slaughter houses when air chilling is used to dry them. However, with poultry, the carcasses are wet and may be packaged soon after slaughter. This is thought to permit extended survival of the bacteria (10). *Campylobacter* spp. can survive for several weeks in ground water, depending on its temperature, and survival is enhanced in the presence of other organisms and in biofilms (11). The survival of *Campylobacter* in the environment through washing and disinfection, and even through repeated washings, disinfection and production cycles (in poultry slaughterhouses) may be due to the bacteria being in biofilm layers (12). *Campylobacter* has been shown to survive for more than one week in biofilms and an increased resistance to disinfection agents has been reported for the bacteria present in such structures (11-14). Snelling *et al*. (15, 16) and Burgess (7) reported that *Campylobacter* spp. are commonly found in communities in biofilms.

High and low temperature stress

1.10 Studies investigating the survival of *Campylobacter* spp. show that the potential increases with decreasing temperature, with survival lasting a few hours at 37^{o}C and several days or longer at 4^{o}C. Even though the organisms are not able to grow below 30^{o}C, probably due to absence of CspA and other cold-shock proteins, metabolic activity has been measured at 15^{o}C and at even lower temperatures (17, 18). A heat shock response has been reported in *Campylobacter* (19) and Zhang *et al*. (20) described differential expression of at least 15 genes in cells grown at 37^{o}C compared to 42^{o}C. These genes are involved in encoding periplasmic or antigenic proteins and the RacR/RacS system is thought to play a role in gene expression at 42^{o}C (21), which may be important in colonisation of poultry.

1.11 *Campylobacter* spp. exhibit a relatively high susceptibility to the effects of freezing (Park, 2002) and this has been attributed to the absence of cold shock proteins (CSPs). A study by Georgsson et al (22) confirmed results from previous studies, reporting that *Campylobacter* levels in broilers were decreased by between 0.7-2.9 $\log_{10}$ units after freezing and 31 days storage. Numbers of faecal coliforms,

often used as indicator organisms, in the same samples remained largely unchanged. The significant die-off of *Campylobacter* is consistent with previous studies (23-25) which reported approximately 2-3 $\log_{10}$ decreases in numbers after freeze-thaw stress, although, again, there is strain-to-strain variability reported with some strains showing higher sensitivity to freezing (26). According to Stern (27) one of the major factors responsible for the reduction in cases of poultry-borne campylobacteriosis in Iceland was the introduction of programme for freezing carcasses from *Campylobacter*-positive flocks. Since then Iceland has mandated a policy for the continued use of this procedure for birds coming from positive flocks. Other countries in Europe, such as Denmark and Norway, have also introduced controls for *Campylobacter* spp. that include freezing of poultry carcasses. Ritz, et al (28) studied the survival of *Campylobacter* on different surfaces of frozen chicken meat but difficulties in modelling the data prevented development of an acceptable predictive model. Three sample types were used; skin, skinned breast meat, and cut muscle surfaces. They were inoculated with high numbers ($10^8/cm^2$) of *Campylobacter* and frozen at -20°C for five weeks. Bacterial numbers were determined weekly using two methods that allowed quantification of uninjured and injured cells. The results showed that the type of chicken surface and the method used to enumerate surviving cells were the most significant sources of variations in the numbers recovered ($P < 0.0001$), much more than the freezing time. Several models were fitted to the count data and found that death rates were nonlinear. Survival was lowest on skin, better on skinned muscle and best on cut muscle. After two weeks, *Campylobacter* numbers plateaued. The authors concluded that sub-lethal injury contributed (an inability to grow on selective media) to variability and the underestimation of bacterial survival and this needs to be taken account in the assessment of *Campylobacter*-associated risk.

1.12 At refrigeration temperatures, numbers of *Campylobacter* show a slow decrease with increased storage time. Bhaduri and Cottrell (29) reported decrease of 0.3-0.81 logs in chicken (skin and ground meat) after 3-7 days storage.

1.13 The decimal reduction time (or *D*-value) for *Campylobacter* spp. is generally reported to be *circa* 1-6.6 min at 55°C and the *z*-value is about 5-6.3°C (30, 31). This is dependent on the heating medium. For example, in milk, *D*-values at 48 and 55°C are 7-13 min and 0.7-1.0 min respectively (32) and at 49, 53 and 57°C, *D*-values in

ground chicken meat were 20.5, 4.9 and 0.8 min respectively (33). In a study using differential scanning calorimetry to determine the mechanism of heat inactivation, Nguyen *et al* (31) concluded that cell death in *C. jejuni* and *C. coli* coincided with the most thermally labile regions of the ribosome. Unusually, cells in exponential phase are reported to be more resistant than ones in stationary phase (34) and at temperatures above 55^{o}C, the kinetics of inactivation are reported to be non-log-linear, with tailing effects observed.

1.14 There are some studies that report unusually large *D* values for *Campylobacter*, when compared to the other publications cited above. De Jong *et al* (35) reported *D* values of *circa* 1.9 min when immersing chicken breast fillets in boiling water, and Bergsma et al (36) reported a value of 1.95 min at a surface temperature ranging from 109-127^{o}C in fried chicken fillets. Although the surface of the breast fillets took some time to reach high temperatures e.g. 85^{o}C in 1 min in boiling water, a significant amount of time was needed to reduce *Campylobacter* to low levels. De Jong *et al*. (35) concluded that chicken meat, product size, challenge temperature or heating rate and cold storage (prior to heating) may have resulted in higher heat resistance than expected. Similar results were also reported for other microorganisms tested in the same study. There are a few other studies that have reported survival of *Campylobacter* when immersing large pieces of poultry in hot water (37, 38) but such studies do not report the data as *D* values. The heating method used in the De Jong *et al*. (35) study is not typical of how chicken is cooked in commercial operations or in the home by consumers. Following the presentation of the work by de Jong (39) and colleagues in 2008, a study from New Zealand (40) reviewed published heat resistance data and concluded that data from New Zealand were in broad agreement with previously published international data and that existing standards for heat treatment practices at manufacturing plants, food services and in homes should be maintained. It is generally well-known that heating in a food matrix results in higher *D* values in comparison to those generated in liquid broth systems and it is reasonable to assume that bacteria inside chicken flesh would survive even better than ones on the surface. However, there are no other published studies that have reported *D* values as high as those by De Jong *et al*. (35) and Bergsma *et al*. (36). Using a similar experimental set-up to that used by Bergsma *et al*. (36), Sampers (41) reported reduction of *Campylobacter* inoculated at

4.5 log cfu/g to below detectable levels after 4 min frying, to an internal temperature of 57.5ºC. When temperatures reached above 50°C (core temperature), *Campylobacter* spp. numbers were below the detection limit (<10 cfu/g). There was no difference between inoculated and naturally contaminated meat preparations. Sampers *et al*. (41) concluded that the consumer information provided by the Belgian Federal Agency for the Safety of the Food Chain, to fry chicken meat until an internal temperature of more than 70°C is reached, is sufficient to ensure *Campylobacter* spp. are eliminated.

1.15 Two important factors about the physiology of *Campylobacter* may help to explain the enhanced heat resistance reported for *Campylobacter* attached to chicken pieces. Recent FSA-funded work (project FS241040) has shown that *Campylobacter* attached to very small pieces of chicken flesh are significantly more heat resistant than cells free in broth, which is in agreement with the earlier work of Blankenship and Craven (33). In addition, unlike most other foodborne pathogens, exposure to refrigeration temperatures does not make *Campylobacter* more heat-sensitive and, in fact, there is a small but significant increase in heat resistance (42, 43). These authors compared *C. jejuni* with *Escherichia coli* to try and explain the different responses to cold with regard to heat resistance. *E. coli* continues to metabolise at low temperature and to achieve this the fatty acid composition of the cell surface membranes is altered to contain more short chain fatty acids to maintain fluidity. This is an advantage in the cold but the more fluid membranes are very sensitive to high temperature. In *C. jejuni* cells exposed to low temperature there is an almost complete and rapid shut down of metabolic activity, as measured by electron transport and the bacterial cells are unable to alter their fatty acid composition and do not adapt to cold exposure. However, their unaltered fatty acid composition is more suited to survival when cells are exposed to high temperatures. This hypothesis is supported by the fact that in *C. jejuni*, the ratio of unsaturated to saturated fatty acids was not significantly different after cold exposure, but it was in *E. coli*.

Low pH stress

1.16 Survival of *Campylobacter* at pH values below 3.0 is poor and absence of epidemiological data linking outbreaks with acidified foods (such as cheese) suggest

that the organism is not able to tolerate the pH and acid levels typically found in these foods. Protection against gastric acid may be afforded by ingestion with buffered foods e.g. milk, or with water, which is rapidly washed-through. In survival studies investigating acid stress, Kelly *et al.* (34) reported mid-exponential cells to be more tolerant of low pH than stationary phase cells, which is not consistent with the behaviour of other bacteria that are generally more resistant in stationary phase. Murphy *et al.* (44) provided an explanation, linking this response to production of a phase-specific extracellular component secreted during growth, contributing to both heat and low pH stresses. In foods, survival at low pH in plain marinade is short-lived, with a > 5 log reduction occurring after 48 h (45). In the presence of meat, however, survival was extended, with organisms surviving for at least 9 days, the author attributing this to the buffering effect of meat.

Oxidative stress

1.17 *Campylobacter* can tolerate reactive oxygen species that may be present in animal or human gastrointestinal tracts. They can respond by producing enzymes such as glutathione, catalase (KatA), peroxiredoxin alkyl hydroperoxide reductase and others (46-48). *Campylobacter jejuni* also produces a superoxide dismutase constitutively, ensuring a basal level of this is present (49). In the previous *Campylobacter* report, we referred to an inherent sensitivity towards oxygen and since then, there have been reports of hyper-aerotolerant *C. jejuni* present on retail poultry meat (50) and these have been shown to survive in poultry meat for extended periods: 5 log reduction over 2 weeks in raw poultry at 4^{o}C compared to 3 days for aero-sensitive strains (51). The frequencies for detection of virulence genes in hyper-aerotolerant *C. jejuni* strains is reported to be significantly higher than in aero-sensitive strains (51). It has also been reported that hyper aerotolerant *C. jejuni* often belong to the same MLST clonal complexes frequently implicated in human infection (50, 51).

1.18 In addition, cells adapted to oxidative stress are reported to be more virulent and have shown enhanced ability to invade Caco-2 cells (52). Culture in low nutrient conditions was the most powerful stressor and affected significantly *C. jejuni* culturability and viability, as well as adhesion to and invasion of Caco-2 cells. Temperature elevation induced a transient growth arrest and a temporary loss of

pathogenic potential, as indicated by impaired adhesion and invasion efficiency of *C. jejuni*. However, bacteria recovered within 24–48 h inside the Caco-2 cells. Oxidative stress neither affected *C. jejuni* growth nor reduced the binding and invasion into Caco-2 cells and short-term exposure to such conditions increased invasion capability and intraepithelial survival of a clinical isolate.

1.19 Yahara *et al* (53) examined the impact of various stages of the poultry production chain on *Campylobacter* populations using Whole Genome Sequencing (WGS) and Genome Wide Association Studies (GWAS). Six hundred *C. jejuni* and *C. coli* isolates from various stages of poultry processing and clinical cases were sequenced. From this population, GWAS was performed with *C. jejuni* ST-21 and ST-45 complexes and identified genetic elements overrepresented in clinical isolates that increased in frequency through the poultry processing chain. Disease-associated SNPs were distinct in these complexes and the function of genes containing associated elements was investigated, demonstrating roles for formate metabolism, aerobic survival, oxidative respiration and nucleotide salvage. This work suggests that poultry processing may select for *Campylobacter* strains better able to cause human infection and thus that there is a link between environmental robustness and virulence. It has been shown using multilocus sequence typing that some *Campylobacter* clonal complexes (such as ST-45) are more frequently isolated from environmental sources such as water, suggesting that strains vary in their ability to survive in the environment (54). This is in agreement with a study by Trigui *et al* (55) who used defined water media, since the composition of tap water is variable. The work showed that some isolates from chicken survived better than others in the defined freshwater model and that survival was affected by temperature and the concentration of NaCl. Comparing the ability of *C. jejuni* to survive in water with other phenotypic properties has shown that survival in water was negatively correlated with auto-agglutination.

1.19 Chlorine (e.g. as sodium hypochlorite) is effective for inactivation of *Campylobacter* spp. in water and biofilms and other disinfectants, such as benzalkonium chloride, peracetic acid are effective at commonly used concentrations (13, 56). However, chlorinated water is not very effective for *Campylobacter* attached to poultry carcasses. Northcutt *et al*. (57) reported that chlorine at 50 ppm

and temperatures up to 54.4ºC had no effect on numbers of the bacteria on chicken carcasses.

Interaction of Campylobacter *with other microbes on its survival and biofilm formation*

1.20 Contaminated water and the natural environment are considered to be important sources of infection with *Campylobacter*. Some studies suggest transmission and survival of this pathogen may be assisted by the free-living protozoa Acanthamoeba. The latter is known to play the role of a host for various pathogenic bacteria, protecting them from harsh environmental conditions (58). Hilbert *et al* (59), using 145 isolates, demonstrated that *C. jejuni* was able to survive for more than 48 hours in aerobic conditions when co-cultured with *Pseudomonas* species. However, co-culture with *Proteus mirabilis*, *Citrobacter freundii*, *Micrococcus luteus* or *Enterococcus faecalis* had no significant impact on survival. Scanning electron microscopy revealed fibre like structures connecting *P. putida* and *C. jejuni* cells. The authors postulated that the bacterium-bacterium interaction might set the basis for survival of *C. jejuni* on chicken meat and thus be the prerequisite step in the pathway toward human infection."

1.21 Biofilms seem to be important for the survival of *Campylobacter* but it is not yet clear if these bacteria can form such structures on their own under natural conditions. Hanning *et al* (60) examined biofilms in poultry house water systems and concluded that the attachment of *C. jejuni* to surfaces was facilitated by pre-established biofilms and survival may also be extended in such communities. However, they also stated that "biofilms do not fully explain long-term survival of culturable *C. jejuni* outside hosts".

1.22 Teh *et al* (61) conducted a mini review on *Campylobacter* biofilms in food-related environments. They concluded that existing studies do not provide strong evidence for biofilm formation (as usually defined) by most *C. jejuni* strains in food-related environments under the combined conditions of atmosphere, temperature and shear that they are likely to encounter. They also concluded that simple attachment to and survival on surfaces and in existing biofilms of other species are more likely to contribute to *C. jejuni* survival in food-related environments.

1.23 Brown *et al* (62) investigated the effects of a chicken meat exudate (chicken juice) on *C. jejuni* surface attachment and biofilm formation. Supplementation of brucella broth with >5% chicken juice resulted in increased biofilm formation on glass, polystyrene and stainless-steel surfaces with both *C. jejuni* and *C. coli* isolates in both microaerobic and aerobic conditions. When incubated with chicken juice, *C. jejuni* was both able to grow and form biofilms in static cultures in aerobic conditions. Electron microscopy showed that *C. jejuni* cells were associated with chicken juice particulates attached to the abiotic surface rather than the surface itself. The authors suggest that chicken juice contributes to *C. jejuni* biofilm formation by covering and conditioning the abiotic surface and is a source of nutrients.

1.24 *Campylobacter* is generally thought to be unable to metabolise glucose due to lack of key glycolytic enzymes. However, Vegge *et al* (63) found that *C. jejuni* subsp. *doylei* has an alternative pathway known as the Entner-Doudoroff (ED) pathway. It was also found in a few *C. coli* isolates. A systematic search by the authors for ED pathway genes in a wide range of *Campylobacter* isolates and in the *C. jejuni/coli* PubMLST database revealed that 1.7% of >6,000 genomes encoded a complete pathway, including both *C. jejuni* and *C. coli* from diverse clinical, environmental and animal sources. In rich media, glucose significantly enhanced stationary phase survival of a set of ED-positive *C. coli* isolates and promoted floating biofilm formation in some of these ED-positive isolates.

Other anti-*Campylobacter* technologies

1.25 Alternative processing technologies are generally effective in destroying *Campylobacter*. A D-value of 0.19 kGy has been demonstrated for *C. jejuni* during irradiation of vacuum-packaged ground pork (64). Lewis *et al.* (65) reported that electron-beam irradiation doses of 1.0 and 1.8 kGy destroyed *Campylobacter* on poultry meat. Ultraviolet irradiation requires a dose of 1.8 mWs/cm^2 to effect a 3-log kill (66). Solomon and Hoover (67) studied the effects of high hydrostatic pressure on *C. jejuni* in chicken meat and demonstrated that pressures of 300-325 MPa could reduce numbers by 2-3 logs and 400 MPa inactivated the organism completely. A different response between exponential and stationary-phase cells has been reported for pressure treatment compared to low pH and heating effects, by

Martinez-Rodriguez and Mackey (68) with early stationary-phase cells being more resistant.

Virulence factors

1.26 Since most disease is associated with *C. jejuni,* more is known about the virulence factors of this species. However, there is still a degree of confusion over the number and specificity of various toxins, due partly to the use of different assays and also to the status of culture filtrates, with some preparations being crude and others being relatively pure. The virulence factors currently identified include heat labile enterotoxin (CJT), cytotoxins including cytolethal distending toxin (CLDT), cytotoxin designated CT, 1-3 galactosyltransferases involved in lipopolysaccharide production, enterocyte-binding factors such as C*ampylobacter* adhesion factor, flagella, outer membrane protein that binds fibronectin, other adhesion factors, and lipopolysaccharide (LPS) required for invasion of intestinal embryonic cells, capsule formation and a virulence plasmid that allows invasion of epithelial cells and also codes for a type IV secretion system. These are described in more detail by Levin (2008) and Bolton (2015). The mechanism of invasion is still not completely understood but the flagella filament is thought to act as a type III secretion system for Cia proteins. Interactions with epithelial cells and role of other virulence factors in *Campylobacter* pathogenesis are reviewed by Poly and Guerry (2008). The invasion of intestinal epithelial cells and presence of cytotoxins are consistent with the bloody inflammatory disease caused by *Campylobacter*.

Viable but non-culturable cells

1.27 *Campylobacter jejuni* is regarded by some to be capable of forming so-called viable but non-culturable (VBNC) cells. Although these cells are metabolically active and show signs of respiratory activity, they are not able to resuscitate by recovery through conventional culturing techniques. The VBNC state has been proposed as a survival strategy or as a moribund condition where cells become progressively debilitated, until they finally ‘die’. Induction of the VBNC state in *C. jejuni* comes about through exposure to sub-lethal adverse environmental conditions, such as prolonged exposure to water or freeze-thaw injury and recovery is affected by passage of the organism through a susceptible host (69-71). This probably reflects the inability of some culturing methods to provide suitable conditions for the

resuscitation of ‘injured’ cells. VBNC *Campylobacter* sometimes form a coccoid shape although non-coccoid VBNCs have also been described (72). There have been examples of outbreaks of campylobacteriosis where cases were observed after attempts to culture the organism from the identified source were no longer positive (73). Treatment of the water supply on one farm also resulted in the disappearance of a particular serotype that had colonised most of the chickens on the farm, although no culturable *Campylobacter* had been isolated from the chicken house water supply (74). The significance of the VBNC remains unclear and reversion of coccoid cells is not easily initiated, requiring very specific conditions. Some studies infer that VBNC cells retain the ability to adhere to and invade intestinal epithelial cells whereas others report a loss of virulence (75, 76). The existence of this state may exert an important influence on considerations in the epidemiology of human and animal campylobacteriosis, and thus should not be ignored.

Antimicrobial resistance

1.28 *Campylobacter* spp. may be exposed to antibiotics during colonisation and carriage in broilers and other farm animals, and there is increasing evidence of indiscriminate use of antibiotics in agriculture resulting in emergence and spread of antibiotic resistant strains (77). In most cases, individuals suffering from campylobacteriosis recover without medical intervention (other than electrolyte and fluid replacement) but in some cases, particularly more severe cases in elderly people, the young, and pregnant women, macrolide, tetracycline or fluoro-quinolone antibiotics may be used. The efficacy of these therapies is increasingly compromised by infections caused by antibiotic resistant strains (78, 79). One of the main mechanisms for development of resistance in *Campylobacter* is thought to be horizontal transfer of gene cassettes, together with modification of pre-existing genes. Several studies have reported on the incidence of antibiotic resistant strains in poultry flocks in Europe (80-82). In the UK, Gormley *et al*. (2010) reported on the AMR profiles of *Campylobacter* isolates from sporadic cases of human infection, retail chicken meat and cattle faeces. Resistance to ampicillin and tetracycline was highest in isolates from human cases (32% and 29% respectively) and retail chicken isolates (25% and 25% respectively), lending further weight to the evidence that chicken is the major source of human *Campylobacter* infection. It is becoming increasingly possible to assess AMR in *Campylobacter*, as in other bacteria, wholly

from WGS data (83). Such data have the advantage of, not only enabling likely antimicrobial resistance to be determined for many antimicrobial agents in a single test, but also permitting the tracking of the evolution, emergence, and spread of such resistance.

Methods for detection/isolation of *Campylobacter*

1.29 There have been many methods developed, and method modifications proposed, for detection of *Campylobacter* in foods. As with the development of methods for other pathogens, media originally used for the isolation of the organism from faeces were employed. Subsequent modifications have been required to enable the detection of low numbers of sub-lethally injured cells in the presence of higher numbers of competitor organisms and this has led to methods based on liquid enrichment prior to selective agar plating with colony identification. Most of the media include ingredients intended to protect *Campylobacter* from the toxic effect of oxygen derivatives. Most commonly used are: lysed or defibrinated blood; charcoal; a combination of ferrous sulphate, sodium metabisulphite and sodium pyruvate; and haemin or haematin.

1.30 *Campylobacter* methods can be divided into two types; those designed to detect the presence or absence of low numbers from a larger amount of food; and those designed to allow the enumeration of the organism. The former tends to require a portion of food to be placed in a selective enrichment broth allowing growth of low numbers, before plating on selective agar permitting the identification of typical colonies. The latter simply require the distribution of a known volume of a diluted food, directly on a selective agar. Typical colonies are then counted.

1.31 While it is normally assumed that detection by enrichment is more sensitive than direct plating, an EU survey reported instances where *Campylobacter* spp. were detected by enumeration but not by enrichment, suggesting that the enrichment method may lead to false negative results (84). It has been suggested that this is related to failure to grow *Campylobacter* sufficiently due to overgrowth of competing microorganisms in the enrichment medium (85, 86). Similar results were noted in a UK survey done over 2007 and 2008 which reported a 50.5% prevalence using an enumeration method but a 33.9% one using a detection method (ACMSF, 2009).

Recovery

1.32 The initial stage of any method will be the recovery of the organism from the food, and this should not be overlooked when determining method efficacy. The importance of the choice of recovery method for maximising the efficacy of recovery of *Campylobacter* from different meat matrices has been highlighted (87). For example, for pork skin samples mechanical pummelling was more effective than swabbing, and peptone water and glucose serum were more effective than demineralised water when comparing recovery fluids used to suspend the organisms following pummelling, whereas no such differences were seen with skinless chicken samples.

1.33 In sampling of poultry, recovery of *Campylobacter* is either by rinsing a portion or the whole carcass, or by taking a sub-sample adding diluents/broths and homogenising.

1.34 The sampling of broiler carcasses has been dealt with in some detail in the draft amendment to Commission Regulation 2073/2005 (88). Here the approach taken is the removal of neck skins, placing in a diluent and homogenising

Enrichment and Isolation

1.35 Several enrichment broths (Bs) for *Campylobacter* have been developed. A study of three of these: Bolton (BB), Campylobacter Enrichment (CEB) and Preston (PB), were compared for the isolation of *Campylobacter* from foods (89). Both BB and CEB were better than PB for the isolation of *Campylobacter* from naturally contaminated foods, although BB yielded more confirmed *Campylobacter* growth than CEB.

1.36 Several agar media have also developed for the isolation of *Campylobacter*. Three of the most commonly used are Butzler agar, Charcoal Cefoperazone Desoxycholate agar (CCDA) and Preston agar. A direct plating method (Karmali agar, biochemical confirmation) and a MPN technique, (Preston broth enrichment, Karmali agar, biochemical confirmation) were developed for the quantification of *Campylobacter* spp. on raw retail chicken legs (90). The direct plating method was considered superior to the MPN technique because it was more rapid, less laborious and more reproducible for certain sample types, but the MPN technique had the

advantage of a lower detection level. Seliwiorstow *et al* (91) have also evaluated a new chromogenic agar for the direct enumeration of *Campylobacter*, which gave equivalent counts to more traditional agars but was more selective reducing the number of competitors able to grow.

1.37 ISO methods (ISO 10272-1 and -2), now give fully detailed standardised approaches for the detection or enumeration of *Campylobacter* from foods (see below).

1.38 Gharst *et al*. (92) have summarised the most effective protocols to isolate *Campylobacter* spp. (mostly *C. jejuni* and *C. coli*) from foods and, in particular, primary poultry products. The recommended temperature for incubation of samples throughout is 42^{o}C, using Bolton broth for enrichment, and transferring enriched samples to CCDA by membrane filters. Addition of blood to plating media aids in differentiation of presumptive colonies with phase contrast microscopy and latex agglutination used for confirmation. Multiplex PCR is the simplest and quickest way to speciate isolates.

Rapid Methods

1.39 In recent years, numerous rapid methods have been developed for the detection of *Campylobacter*, some of which have been evaluated for application with foods. However, very few of these methods have been commercialised, which probably reflects the fact that the food industry is not performing high levels of testing for *Campylobacter* and the market for rapid method test kits is therefore small. The reason for the lack of testing by the food industry is probably due to several factors including: the organism does not grow in food under most normal storage conditions; it does not survive well and is relatively easily controlled in processed foods; it is prevalent in raw foods where the ultimate critical control is in the hands of the consumer; and the organism is fastidious and its detection and maintenance in the laboratory are not easy.

1.40 Of the rapid methods that have been commercialised, validated and certified (through ISO 16140 or AOAC Research Institute), a number are alternative agars that have been developed to help differentiate *Campylobacter* from competitors when doing isolation plates. There are two immunoassays, and four molecular based

detection systems (PCR or isothermal amplification). Except for the agar-based media, all are directed at detection rather than enumeration of the organism.

1.41 There has been much research into methods that simplify the confirmation and identification of *Campylobacter*. Some of this work has led to the development of commercially available systems. The API Campy kit is commercially available for the differentiation of *Campylobacter* spp., although identification of species within the family Campylobacteraceae using standard biochemical tests can be problematical because of the variability and atypical reactions of some strains (93).

1.42 A more recent approach to species identification or confirmation of isolated colonies, has been the use of Matrix Assisted Laser Desorption Ionisation Time of Flight mass spectroscopy (MALDI-ToF). Bessede *et al* (94) reported good results when comparing MALDI ToF with PCR based method for identifying *Campylobacter* species with accuracies of 99.4% to 100%. The authors also reported the very fast analysis time (2 minutes for an isolated colony identification). The MALDI approach has also been noted in Public Health England's (PHE) Method for Identification of *Campylobacter* species (95). They note that MALDI can provide rapid and accurate species - level identifications for *C. jejuni* and *C. coli*; as well as emerging *Campylobacter* species such as *C. lari, C. fetus, C. hyointestinalis*, *C. upsaliensis, C. sputorum*. They do note that taking colonies directly from CCDA may adversely affect spectra, and that a sub culture onto a non-selective agar may be required before MALDI is used for *Campylobacter* identification. The use of a validated MALDI approach to identification is also noted with the ISO 10272-1 and -2 methods that were published in 2017.

Reference Methods

1.43 There are several standardised reference methods available for detection of *Campylobacter* from foods. These will usually be ones required by legislation or specifications.

1.44 ISO 10272-1:2017. Microbiology of the food chain - Horizontal method for detection and enumeration of *Campylobacter* spp. - Part 1: Detection method. This part of the ISO method concentrates on the detection of *Campylobacter* from foods. It gives three approaches to enrichment (96):

A: Detection of *Campylobacter* by enrichment, in samples with low numbers of the bacteria and low level of background microflora and/or with stressed *Campylobacter* spp. This uses Bolton Broth as the enrichment system, followed by isolation on mCCDA plus one other selective agar (this agar should be based on different selectivity principles to mCCDA)

B: Detection of *Campylobacter* by enrichment, in samples with low numbers of *Campylobacter* spp. and high level of background microflora. This uses Preston Broth as the enrichment system, followed by isolation on mCCDA.

C: Detection of *Campylobacter* by direct plating on mCCDA, in samples with high numbers of the bacteria (note: this direct plate method is for detection purposes, not enumeration which is covered by ISO 10272-2).

1.45 ISO 10272-2:2017. Microbiology of the food chain -- Horizontal method for detection and enumeration of *Campylobacter* spp. -- Part 2: Colony-count technique. This part of the ISO method is centred on obtaining a count of *Campylobacter* from a sample and is based on the direct plating of a sample or a dilution of it onto mCCDA (96).

1.46 United States Food and Drug Administration (97) method for the detection of *Campylobacter* from food and water Bacteriological Analytical Manual. Chapter 7. *Campylobacter:* the US FDA method provides a range of enrichments, the use of which depends on the sample type. Generally, most enrichments will utilise Bolton Broth at some point followed by isolation on either Abeyta-Hunt-Bark (AHB) agar or mCCDA.

1.47 United States Department of Agriculture Food Safety Inspection Service (98) Direct Plating and Enrichment using conventional methods (both enrichment and direct plating) for isolation and identification of *Campylobacter* from poultry rinse, sponge and raw product samples, but also details the use of a commercially available PCR based detection method.

1.48 The USDA FSIS method utilises Bolton Broth for enrichment-based methods, followed by plating on Campy-Cefex plating medium.

1.49 Within the EU the methods of choice will be those detailed by ISO, which will automatically become European Standards (CEN) and be transferred into National Standards by all Member States. These ISO methods are British Standards. The importance of the use of standardised methods is that they will ensure better data comparability between laboratories, production sites and countries. Recent proposed changes to legislation within the EU will introduce a process hygiene criterion for *Campylobacter* on broiler carcasses (by amending Annex 1 of Commission Regulation 2073/2005). This amendment will require the use of ISO 10272-2 to enumerate the organism on carcasses. However, Article 5 of EC 2073/2005 would allow other enumerative methods to be used as long as they were validated using protocol defined in ISO 16140 (Validation of microbiological test methods) and certified by a third party, thus indicating that they gave equivalent results to the reference method.

1.50 For epidemiological studies, molecular typing techniques are described in more detail in the following chapter.

Conclusions and recommendations

1.51 Increased understanding of mechanisms of stress response and biology of *Campylobacter* has revealed a number of alternative mechanisms that allow the bacteria to survive under stress conditions but this has yet to lead to development of new strategies for improved control. We recommend that research is undertaken to determine the impact of genetic diversity in *Campylobacter* spp. on the ability of the bacteria to survive in and respond to hostile conditions found in the poultry food chain.

1.52 As the previous ACMSF report concluded*, Campylobacter* spp. are sensitive to low pH and low a_w stress conditions (e.g. desiccation), and commonly used disinfectants, dying off relatively rapidly compared to other foodborne bacterial pathogens. In addition, alternative processing technologies such as irradiation, and high-pressure processing are generally effective in destroying *Campylobacter*.

1.53 Studies investigating tolerance to aerobic conditions that have been published since the last ACMSF *Campylobacter* report indicate that some strains can become hyper aerotolerant, surviving much longer than aero-sensitive strains and there is

some suggestion that these may be more virulent than aero-sensitive strains. This suggests that *Campylobacter* spp. may not be as fragile as previously thought.

1.54 There is little evidence of biofilm formation by *Campylobacter* spp. Simple attachment to and survival on surfaces and in existing biofilms of other species are more likely to contribute to *C. jejuni* survival in food-related environments.

1.55 There is some evidence supporting the view that survival of *Campylobacter* may be assisted by other organisms such as *Acanthamoeba* and *Pseudomonas* species. Further work in this area is required to determine the significance of these findings.

1.56 Most studies investigating heat resistance report relatively small *D* values, indicating that *Campylobacter* spp. are more heat sensitive compared to other infectious bacteria, at least in laboratory media. There is general agreement that when *Campylobacter* spp. are attached to chicken meat, higher *D* values are reported. A small number of studies published since the last ACMSF report that used large pieces of poultry immersed in boiling water or fried, indicate unusually long times would be required for complete destruction. No other studies have reported these unusually high heat resistance values. Before considering the impact of the two heat resistance studies reporting unusually high *D* values when cooking chicken meat, and considering changes to cooking instructions for meat processing facilities, catering or cooking in the home, further work should be carried out to determine if these results are reproducible by other workers, and in this further work, it is critical to accurately measure the coldest point in the meat being cooked. Depending on results, further research could be carried out to establish the mechanisms underlying the markedly increased heat resistant of *Campylobacter* cells attached to surfaces and particularly on chicken skin and muscle.

1.57 **It is recommended that the public health significance of the VBNC state is explored further.**

1.58 It is becoming increasingly possible to assess antimicrobial resistance in *Campylobacter*, as in other bacteria, wholly from WGS data. We therefore recommend that determination of AMR from WGSs becomes accepted as standard for *Campylobacter* (See also Chapter 2).

1.59 Methods for recovery of *Campylobacter* are now well established, with standardised reference methods available for detection of the bacteria from foods. These methods will usually be those required to be used by legislation or specifications and will ensure better data comparability between laboratories, production sites and countries. Developments in molecular approaches allow rapid characterisation of different *Campylobacter* species and MALDI-ToF has also been successfully applied for this purpose.

Chapter 2: Campylobacter *genetics and genomics*

The development of genetic approaches to studying Campylobacter

2.1 The past twenty years of genetic analysis have been dominated by the application of continually improving and increasingly cost-effective means of nucleotide sequence determination (sequencing). Over this time, sequencing has moved from being an approach used rarely for individual genes of a few isolates, and mainly for research purposes, to one which is employed routinely in a wide variety of applications including routine isolate characterisation. It is now possible to determine the complete or near complete genome sequences (WGSs) of tens of thousands of isolates and, more recently, whole communities or ecosystems. Increasingly, nucleotide sequence-based approaches are being integrated with genetic and biochemical techniques to explore the phenotypes of bacteria and their evolution. Very large-scale sequencing studies of *Campylobacter* isolates have provided major insights into the epidemiology of campylobacteriosis, as well as providing information on the population structure and evolution of different phenotypes within the genus.

Nucleotide sequence-based isolate characterisation and 'sequence-based typing'

2.2 An ideal isolate characterisation, or typing, system will be applicable across the bacterial domain and able to differentiate closely related isolates, in addition to resolving more distant genealogical relationships. It should also be rapid, simple, scalable, reproducible, and have a high level of typeability (*i.e.* success rate across isolates or samples examined). The protocols, reagents, and data generated should be portable and accessible, and interpretation of the results objective. It is essential that the method(s) adopted are widely agreed on and adopted by the scientific community (99, 100). Although no current typing system currently meets all of these criteria, sequence-based techniques can meet many of them (100). Several sequence-based typing techniques have been developed for *Campylobacter*, including single locus, multilocus, and WGS-based methods (101).

Single locus sequence typing

2.3 The single locus methods most commonly used for *Campylobacter* sequence typing index variation in highly variable surface antigen genes, for example *flaA* and *flaB* (which encode highly variable flagellin proteins) and *porA* (which encodes the major outer membrane protein). These techniques were developed for typing *C. jejuni* and *C. coli* isolates, and involve PCR amplification of the ~320 bp short variable region (SVR) of *flaA* or *flaB* (102), or a ~630 bp internal fragment of *porA* (103), followed by direct nucleotide sequencing. The resulting nucleotide sequences are compared to variation catalogued in public databases, such as the *C. jejuni*/*coli* PubMLST database (http://PubMLST.org/campylobacter) (104), and novel variant sequences are assigned unique nucleotide and peptide allele numbers in order of their discovery. As of July 2017, 1,661 *fla*-SVR nucleotide and 370 peptide allelic variants had been defined in the PubMLST database, and 2,196 and 2,011 *porA* nucleotide and peptide allelic variants, respectively.

2.4 Both *fla*-SVR and *porA* typing have been employed to study the epidemiology of human campylobacteriosis, and, in combination with other typing methods, have been applied to investigate outbreaks (102, 103, 105-111). A major drawback of single locus typing schemes is, however, that relationships among isolates can be distorted by horizontal genetic transfer (HGT) that results in intra-gene recombination. This is a consequence of the fact that *Campylobacter* are naturally competent for DNA uptake (112). Intra- and inter-species HGT are well established as drivers of genetic diversity, particularly among *C. jejuni* and *C. coli* (113, 114). For example, *fla*-SVR and *porA* alleles are shared between *C. jejuni* and *C. coli* (109, 111), while intraspecies and intragenomic HGT in the *fla* genes has also been observed. These effects cause closely related isolates to appear distinct when examined solely at single loci (115, 116). As a result, *fla*-SVR and *porA* typing are best used in conjunction with methods that interrogate multiple genetically stable loci, such as multilocus sequence typing (MLST).

Multilocus sequence typing (MLST)

The development of Campylobacter *MLST schemes*

2.5 MLST is a generic typing technique that was first developed for the human pathogen *Neisseria meningitidis* (117) and has since been adapted for over 100 taxa. The majority of MLST schemes have been developed for pathogenic members of the bacterial domain, although it is suitable for a wide range of applications (118). MLST data are made widely available via several websites (http://pubmlst.org/; http://www.mlst.net/; http://bigsdb.web.pasteur.fr/; http://mlst.warwick.ac.uk/mlst/). The method is based on the population genetics framework used previously for multilocus enzyme electrophoresis (MLEE), which assesses variation in genes that encode essential metabolic functions, referred to as housekeeping genes (118). In MLEE, genotypic allelic variants are inferred based on differences in the electrophoretic mobility of isozymes of the enzymes investigated (119). Similar to MLEE, MLST indexes nucleotide sequence diversity in housekeeping genes, which are considered to be under stabilising selection for conservation of metabolic function, enabling the study of genealogical relationships within a species group or among related species (118). In conventional MLST, internal fragments of 400-600 bp are amplified from 6-10 housekeeping genes by PCR and sequenced directly. The resulting nucleotide sequences and isolate provenance data are submitted to an appropriate online database, compared to existing sequences available for each locus, and assigned unique allele numbers in order of discovery. Together, these numbers form an isolate's allelic profile or sequence type (ST), which is also summarised as a unique, arbitrary number (117).

2.6 To describe relationships among isolates, MLST STs are grouped into clonal complexes, which are sets of isolates with related STs, which correspond to genotypes that are likely to have descended from a common ancestor. In the case of *Campylobacter* MLST schemes, clonal complexes are defined by identifying a central ST (as a surrogate for genotype) and its related STs (genotypes), those which share most loci. Central STs occur at a high frequency, persist over time, and occupy a central position when STs from the population of interest are analysed using heuristic algorithms. Members of a particular clonal complex share four or more alleles with the central ST. In addition to describing relationships among

isolates based on allele designations, STs, and assignment to clonal complexes, they can also be defined by phylogenetic analyses of the underlying nucleotide sequences. The choice of analytical approach is dictated by the population structure of the organism of in question, with allele-based approaches appropriate for studying populations with high levels of inter-species HGT and phylogenetic analyses for clonal populations with low inter-species HGT (118, 120).

2.7 The first *Campylobacter* MLST scheme was developed for *C. jejuni*, including *C. jejuni* subsp. *doylei,* in 2001 (121). The scheme was based on seven *C. jejuni* loci: *aspA*, *glnA*, *gltA*, *glyA*, *pgm* (now known to be *glmM*), *tkt*, and *uncA* (also referred to as *atpA*) and has since been extended to include *C. coli* (109, 122). Two other MLST schemes were developed for *C. jejuni* (123, 124), but these have not been widely used (120).

2.8 As MLST is based on housekeeping genes that are under stabilising selection, it is well-suited to long-term epidemiological studies (118). To increase the discriminatory power of MLST for studies of short-term *Campylobacter* epidemiology, the *C. jejuni/C. coli* scheme has been supplemented with additional loci, the antigen genes *flaA, flaB*, and *porA* (103). These genes are under diversifying selection, probably because of immune selection from host responses, and are therefore more variable. As for *fla*-SVR and *porA*, *C. jejuni* and *C. coli* MLST data are submitted to the *C. jejuni/coli* PubMLST database. The database is used widely and contains at least one representative of each ST, providing a comprehensive summary of known MLST diversity for these species. As of July 2017, the *C. jejuni/coli* database contained 52,727 isolates, corresponding to 8,589 unique MLST profiles and 44 clonal complexes.

2.9 Subsequent to the development of the *C. jejuni*/*C. coli* MLST scheme, additional schemes were introduced for: *Campylobacter concisus*; *Campylobacter curvus*; *Campylobacter fetus; Campylobacter helveticus*; *Campylobacter hyointestinalis*; *Campylobacter insulaenigrae*; *Campylobacter lanienae*; *Campylobacter lari*; *Campylobacter sputorum*; and *Campylobacter upsaliensis* (122, 125-127). These schemes are also hosted on the PubMLST website, and are accessed *via* the non-*jejuni*/*coli* database. The

non-*jejuni*/*coli* database is smaller than the *C. jejuni*/*coli* database: as of July 2017, it contained 349 isolates and 837 MLST profiles across nine schemes.

The impact of MLST on our understanding of Campylobacter *epidemiology, population biology, and evolution*

2.10 As reviewed previously (120), MLST substantially advanced the understanding of the epidemiology, population biology, and evolution of members of the *Campylobacter* genus, especially *C. jejuni* and *C. coli*. The success of MLST depended on concurrent advances in high-throughput sequencing approaches and information technology. The former enabled MLST studies of large isolate collections and the latter made MLST data portable and accessible through online public databases (118). MLST-based studies of *C. jejuni* and *C. coli* have demonstrated that, although genotypes causing human disease are highly diverse, they overlap both nationally and internationally (103, 128-134) and are distinct from those of *C. jejuni* subsp. *doylei* (135). Although *C. jejuni* from high-income countries are genetically similar, isolates from Curaçao, a Caribbean island, were distinct from those from more industrialised countries, including the UK, Canada, and Australia (103, 136). This suggested that the distribution of *Campylobacter* lineages may differ in high, low, and middle-income countries, perhaps because of differences in food production, distribution and consumption.

2.11 The availability of large MLST datasets, including data derived from representative collections of isolates obtained from of a variety of food animals, facilitated the development of formal genetic methods to attribute human disease isolates to sources of infection (137-141). These methods have indicated consistently that retail chicken meat is a major source of campylobacteriosis in many high-income countries (134, 140-146). Attribution studies have also identified seasonal differences in the relative abundance of certain genotypes, as well as associations with age, gender, population density, and travel (134, 143, 145, 147). Source attribution has been used to monitor the effects of interventions designed to reduce the incidence of human disease, as illustrated by the disease surveillance conducted in New Zealand following the introduction of a series of interventions in the poultry industry. This detected a 74% reduction in cases attributed to poultry,

approximately halving the national human disease burden (148). (148). This reduction has persisted. (149).

2.12 A detailed understanding of the ecology of *Campylobacter* is a prerequisite for the development of effective intervention strategies, and MLST-based studies have revealed associations between certain lineages and particular animal hosts. For instance, clonal complex ST-257 is over-represented among isolates obtained from chicken sources and ST-61 in bovines, while others, such as the ST-21 clonal complex, are regularly isolated from a broad range of hosts and are considered 'generalist' or perhaps 'agriculture-associated' genotypes (120). The fact that the signal of host-association of certain genotypes obscures intercontinental geographic signals implies that there is a link between the global distribution of food and the international dissemination of *Campylobacter* genotypes (150, 151). Taken together, the results of MLST-based epidemiological investigations in humans and animals suggest that diet, agricultural practices, and food distribution networks all impact on the global epidemiology of *C. jejuni* and *C. coli* (120).

2.13 The population structures of both *C. jejuni* and *C. coli* are strongly influenced by HGT, but are distinct. Whereas the *C. jejuni* population comprises a large number of clonal complexes, which have little evidence of a clonal frame among them (106, 152), the *C. coli* population forms three relatively isolated clonal groups, known as clades 1, 2, and 3 (109, 113, 153). HGT within clades and clonal complexes is relatively frequent, but is less common among them. The majority of *C. coli* human disease isolates belong to clade 1, as do most agricultural isolates, but in contrast, isolates from clades 2 and 3 correspond predominantly to environmental samples and are rarely associated with human disease (151). *Campylobacter* are naturally competent for DNA uptake (112), and MLST studies have provided evidence of progressive introgression, the introduction of alleles from one species into the gene pool of another due to intra-species HGT, from *C. jejuni* to *C. coli*, suggesting that the species are converging, or 'despeciating', having initially diverged from a common ancestor(113, 154, 155). It has been postulated that agriculture is driving this process, as it has generated a new niche in which *C. jejuni* and *C. coli* overlap (113, 153).

2.14 Studies of other *Campylobacter* species have included smaller numbers of isolates compared to those of *C. jejuni* and *C. coli*, but have also provided insights into their epidemiology and population structures. For example, although *Campylobacter fetus* subsp. *fetus* has been isolated from a variety of host sources and *Campylobacter fetus* subsp. *venerealis* appears to be host-restricted to the genital tract of cattle, and these species were found to be genetically homogeneous by MLST (125). In contrast, *C. lari*, *C. upsaliensis*, and *C. helveticus* are genetically more diverse, with HGT detected among MLST loci in *C. jejuni* and *C. lari*, and *C. upsaliensis* and *C. helveticus* (122).

Genomic approaches to the investigation of Campylobacter

2.15 Although MLST has led to important advances in our knowledge of *Campylobacter*, especially its epidemiology, the technique is considered labour intensive and expensive, which has prohibited its widespread adoption in routine application (101). Given the very high diversity of *Campylobacter* isolates, conventional seven-locus MLST, whilst excellent at assigning isolates to clonal complexes and also in attribution analyses, can lack sufficient discriminatory power for outbreak investigations (120). As discussed, the inclusion of additional loci, specifically highly variable antigen genes such as *fla* and *porA*, provides a level of discrimination that can resolve outbreaks (103); however, this increases labour requirements and costs by addition of further loci. As a result, there remained a need for highly discriminatory, portable, and objective typing techniques that could be easily incorporated into the clinical microbiology laboratory, which is increasingly met by the application of WGS approaches.

Whole-genome sequencing

2.16 Studies published from 2010 onwards have demonstrated the potential of WGS for use in a wide range of applications in clinical microbiology, including for diagnosis and public health. The availability of WGS data represented a step change in the quantity and discriminatory power of sequencing data available routinely which can be used for short- and long-term epidemiological investigations, as well as for very high-resolution studies of bacterial population genetics and evolution.

Next-generation sequencing platforms

2.17 The bacterial genomic era began in the mid-1990s and was based on Sanger (chain terminating, dideoxy-nucleotide based) sequencing technology (156, 157). The first 'next-generation sequencing' (NGS) platforms were introduced in 2005 (157, 158) and competition among manufacturers resulted in the very rapid development of these platforms, as well as enhancements to existing technologies (159). Consequently, sequencing capacity and associated costs improved, making bacterial WGS increasingly accessible (160, 161). In July 2017, the Sequence Read Archive (SRA) (https://www.ncbi.nlm.nih.gov/sra) at the National Centre for Biotechnology Information (NCBI), one of the main repositories for raw NGS data, contained 575,871 records corresponding to bacterial DNA sequences with the Illumina platforms dominating at that time, accounting for approximately 92% of sequences.

2.18 NGS platform choice should be guided by the intended application and practical considerations. Up to the time of writing, most studies on the genomic epidemiology of bacteria were retrospective, having been carried out primarily by academic institutions. In this context, samples were frequently batched (or multiplexed) and sequenced on high-capacity, high-throughput NGS platforms such as the Roche/454 GS FLX+, the RS II (Pacific Biosciences), and the Illumina Genome Analyser and HiSeq machines. However, due to their significant initial cost, the necessity of high-throughput for cost efficiency, and their large laboratory footprints, these platforms were best suited to sequencing centres and core facilities. With an increasing emphasis on real-time genomic epidemiology, there was a growing need for rapid, cost-effective near-patient sequencing instruments with smaller capacity. Compact bench-top machines, such as the Roche/454 GS Junior, the IonTorrent Proton, and the MiSeq from Illumina met this need. The Oxford Nanopore MinION, which is pocket-sized, represented the first portable NGS platform and the potential of this device for real-time outbreak investigations was demonstrated during the 2014 Ebola and 2016 Zika epidemics and a *Salmonella enterica* outbreak in a UK hospital (162-164).

Current status of global genomic databases for *Campylobacter*

2.19 When the genome sequence of *C. jejuni* NCTC 11168 was completed in 2000 (165), the nucleotide sequence and annotations were submitted to members of the International Nucleotide Sequence Database Collaboration (INSDC) for storage and dissemination. As more bacterial genomes became available in the early 2000s, INSDC databases continued to be important nucleotide sequence repositories, but smaller, bespoke databases began to emerge. Forerunners such as xBase (166), the UCSC Microbial Genome Browser (167), and the BacMap Genome Atlas (168) collated genomes of *Campylobacter* and other bacterial species, facilitating genome browsing and simple comparative analyses. However, these databases contained relatively small numbers of *Campylobacter* genomes. In the first decades of the 21st Century, developments in NGS technologies and concurrent decreases in associated costs (160) resulted in a major increase in the sequencing of bacterial isolate genomes, including for *Campylobacter*. This was reflected in deposits to the SRA, which contained 26,693 *Campylobacter* genomic records in July 2017, most of which were released after 2011 (Figure 2.1). Use of the raw (that is, unassembled) sequencing data stored in the SRA was limited to those laboratories with bioinformatics resources and expertise; therefore, public databases with assembled bacterial genomes became important resources. As of July 2017, 192 complete and 1,957 draft (assembled but not 'finished') *Campylobacter* genomes, corresponding to at least 27 species, were available through the INSDC member databases (Figure 2.1).

2.20 The increasing availability of bacterial WGS data catalysed the development of specialist online genomic databases. These databases linked provenance, laboratory, and nucleotide sequence data, but differed with respect to size and analytical capabilities. Key databases were queried for *Campylobacter* genomes in July 2017 (summarised in Table 2.1), and the analysis tools that were available at that time are described briefly here. Ensembl Bacteria published annotated bacterial genome sequences (169); however, most analysis tools had not been applied to members of the *Campylobacter* genus at the time of writing. The Joint Genome Institute (JGI) Genomes OnLine Database (GOLD) catalogued sequencing projects and linked associated metadata (170), interfacing with the JGI Integrated Microbial

Genomes and Microbiome Samples (IMG/M) website. In addition to storing annotated genomes and microbiome data, IMG/M facilitated searches for genes and functions of interest, as well as genomic analyses, such as synteny comparisons, homologue detection, average nucleotide identity, and genome clustering (171). Similar to IMG/M, the Pathosystems Resource Integration Center (PATRIC) was used by biomedical researchers to store and analyse bacterial genomes (172). PATRIC could be used to assemble and annotate genomes, compare protein families, and explore metabolic pathways. Following the success of the PubMLST databases for storage, dissemination, and analysis of MLST data, the underlying software was adapted for WGS data (104) (details below). The Ribosomal Multilocus Sequence Typing (rMLST) (173) and *C. jejuni/coli* PubMLST databases contained the largest number of *Campylobacter* genomes (Table 2.1). PubMLST databases were distinct from the other genome databases described here because they facilitated a broad range of gene-by-gene analyses, enabling users to study the epidemiology, population biology, and evolution of *Campylobacter*.

Analysis of genomic data

2.21 The first studies to use WGS to investigate the epidemiology of bacterial pathogens employed an approach known as read mapping, which was originally developed for the analysis of human genome resequencing data. In this approach, the short-read sequences generated by NGS were aligned to a reference genome to identify single nucleotide polymorphisms (SNPs) between the reference and the sequence examined. These were used to construct phylogenies and visualise the relationships among collections of query isolates with respect to the reference genome or genomes (174). Read mapping has been applied to study the short- and long-term epidemiology of several clinically important bacteria, including *Mycobacterium tuberculosis*, *Staphylococcus aureus, Streptococcus pneumoniae, Clostridium difficile*, *Vibrio cholerae*, *Salmonella* Typhi and *Shigella flexneri* (175-185). An important drawback of the read mapping approach, however, is that it cannot be used if an appropriate reference genome is not available and regions absent from the reference genome but present in the query genomes are not included in the analysis (174).

2.22 As the sequence read lengths attainable by NGS technologies increased from tens of base pairs to hundreds, it became possible to assemble draft genomes *de novo* from these data, generating 'contiguous sequences' or 'contigs' based on consensus sequences assembled from overlapping short sequence reads (186). This allowed for analysis of WGS data without the requirement for a reference genome. Numerous genome assembly methods have been developed, most of which make use of the mathematical approach of de Bruijn graphs (187), including VELVET (188), EULER-SR (189), ABySS (190), SOAPdenovo (191), SPAdes (192), and many others. Among these tools, VELVET was one of the first-developed and most widely employed programs, as it was well-suited for use with Illumina data (174, 186); however, since its release in 2012, SPAdes has become an increasingly popular alternative to VELVET for the assembly of microbial genomes. The Genome Assembly Gold-standard Evaluation for Bacteria (GAGE-B) study found that SPAdes typically outperformed VELVET, particularly with respect to MiSeq data (193).

2.23 Once assembled, draft genomes can be aligned using programmes such as MUMmer (194), Mugsy (195), or Mauve (196, 197) and SNPs extracted for phylogeny reconstruction, but these approaches work best with small datasets. The Roary pan-genome pipeline was developed for use with large microbial datasets, identifying core and accessory genes and generating nucleotide sequence alignments that can be used to reconstruct phylogenies (198). As HGT distorts phylogenetic signals, putative regions of recombination are often removed prior to generating a phylogeny, most commonly using the program Gubbins (199). Regions of recombination were also frequently removed in read mapping analyses, but the process has been shown to exacerbate the misrepresentation of branch lengths (200). An alternative approach is to use ClonalFrameML, which attempts to detect recombination and account for it when generating a phylogeny (201). It is, however, problematic that, like read mapping, these programs are computationally intensive and often do not scale well when working with large datasets. Furthermore, the approaches described here require extensive computational resources and bioinformatics expertise. The gene-by-gene approach implemented using web-based platforms, such as the Bacterial Isolate Genome Sequence Database (BIGSDB), represents an alternative approach for the rapid, consistent analysis of WGS data without relying on complex bioinformatics pipelines (100).

2.24 The gene-by-gene approach defines relationships among isolates based on the comparison of alleles assigned for a particular set of loci, as in MLST. There is consequently no requirement to identify and exclude those changes that are due to HGT, and analyses can be carried out using allele numbers or nucleotide sequences as appropriate. The gene-by-gene approach is flexible, in that the number of loci included in an analysis can be adjusted based on the level of resolution required. One of the strengths of this method is that it can be used to relate typing data to nomenclature. Smaller sets of loci, such as the 16S rRNA gene or the MLST loci, can be used for low resolution typing, thereby identifying isolates to the genus and species-levels. The number of loci analysed can then be increased when higher resolution is required: ribosomal MLST (rMLST) can be used to identify isolates up to the lineage level (202), core-genome MLST (cgMLST) can be used to examine relationships among related yet distant groups of isolates, and whole-genome MLST (wgMLST), encompassing all genes shared among isolates, can be used to distinguish closely related strains and clones (100).

2.25 The gene-by-gene approach has been implemented using the open-source web-accessible BIGSDB software (100, 104). BIGSDB comprises two databases, the isolate database and the sequence definitions database. The isolate database links provenance and phenotype data to nucleotide sequences, while the sequence definitions database contains all known alleles for user-defined genes of interest. By means of a process known as scanning and tagging, the contigs in the isolate database are searched for sequences similar to the indexed loci (scanned) using the BLAST algorithm. When an existing allele is identified, the sequence is marked, or tagged in the isolate database, indicating its position in the genome and allele designation. New alleles are first added to the sequence definitions database, and then the isolate is re-scanned and tagged in an iterative process (104).

2.26 In addition to storing data and annotating genome sequences, BIGSDB also includes analysis tools, such as the GENOME COMPARATOR module. GENOME COMPARATOR can be used to compare isolates using user-defined loci, which can be grouped into schemes useful for typing or taxonomy, or to aid functional annotation. Alternatively, isolates can be compared to an annotated reference genome. In both cases, the resulting allelic profiles are converted to a distance matrix, which is

automatically visualised as a NeighborNet graph (network) using SplitsTree (150, 203-205). GENOME COMPARATOR also produces a list of variable loci and, if requested, additional outputs, such as genetic distances and nucleotide sequence alignments for use in further analyses (100, 104). Since its development, the gene-by-gene approach has been used to study the epidemiology of several bacterial pathogens, including *Campylobacter* (100, 206), *Neisseria* (173, 191, 207-210), *Escherichia coli* (211), *Streptococcus pneumoniae* (212, 213), *Listeria monocytogenes* (214, 215), *Enterococcus faecium* (216) and *Elizabethkingia anopheles* (217).

2.27 Core genome MLST (cgMLST) schemes define those loci that are found in most isolates in a given collection. When used for high-resolution bacterial typing of isolates characterised by WGS data generated with current methodologies, such cgMLST schemes cannot be absolute, *i.e.* contain only those loci found in all isolates. Rather, they have to be defined pragmatically for the following reasons: (i) all isolates are likely to be mutants at a small number of loci and including only those functionally present in every isolate will ultimately reduce the cgMLST loci to an unrealistically low number; (ii) the use of draft genomes, even those of high quality, will also result in the incomplete assembly, or even complete loss, of certain loci in some samples for technical reasons; (iii) problematic loci, such as paralogous loci (closely related genes that occur at different loci) or those that do not assemble reliably will need to be removed even if they are present in all isolates. Consequently, the definition of a cgMLST scheme is dependent firstly on the subset of isolates to be examined, secondly on the distribution of loci within that collection, and finally on the removal of loci that may cause inconsistent results. As cgMLST schemes are simply lists of loci, associated with catalogues of the nucleotide sequences defining alleles at each of those loci, it is possible to define multiple cgMLST schemes for different purposes and to refine them as knowledge develops. To maintain consistency of typing over time, it is necessary that these schemes have clearly defined names and are constructed such that it is possible to perform comparable analyses over time and across studies.

2.28 A cgMLST scheme has been proposed as a universal typing approach to isolates from human campylobacteriosis isolates (218). This ‘Human

Campylobacteriosis cgMLST Scheme (v1.0)' includes 1,343 loci, the allelic profiles of which can be assigned to core genome sequence types. These loci were a subset of the 1,643 identified in the reannotation of the genome sequence of *C. jejuni* isolate NCTC 11168 (219). As this cgMLST scheme is intended to characterise isolates from human campylobacteriosis (*i.e.* both *C. jejuni* and *C. coli*, but specifically those genotypes associated with human disease), 2,472 representative United Kingdom campylobacteriosis isolates were used to establish the scheme, comprising high-quality draft genome sequences from 2,207 *C. jejuni* and 265 *C. coli* isolates. Loci for inclusion in the scheme were chosen based on their frequency distribution in the dataset with those loci present in >95% of draft genome sequences included. Validation of the scheme was undertaken with 1,478 additional high-quality draft genome sequences, 99.5% of which contained >95% of the 1,343 loci. This scheme was further validated by the examination of an outbreak previously investigated by WGS (218, 220).

Insights from WGS analyses of Campylobacter

2.29 Advances in NGS, in conjunction with the large volume of publicly available genomic data, have facilitated studies of *Campylobacter* genetics and genomics that were previously not possible. Some early insights into the epidemiology, population biology, evolution, and functional genetics of *Campylobacter* arising from WGS studies are noted here.

Implications for epidemiology

2.30 Due to the high initial cost of genome sequencing, early investigations into the suitability of WGS for *Campylobacter* epidemiology typically focused on small datasets from suspected point source outbreaks. These studies established the utility of WGS for outbreak investigations and contact tracing. For example, WGS was used to investigate retrospectively several *C. jejuni* and *C. coli* outbreaks, including: milk- and water-borne outbreaks in Finland (221, 222); a milk-borne outbreak in the UK that was caused by pasteurisation failure (220) ; a water-borne outbreak in Canada (223); and large-scale chicken liver pâté-associated outbreaks in Australia and Sweden (224, 225). All but one of these investigations reported limited genetic diversity among outbreak isolates, which differed at approximately 15 or

fewer loci when analysed at the cgMLST or wgMLST levels, with most variation occurring in homopolymeric tracts. In contrast, multiple STs, and even species, were recovered from the Australian chicken liver pâté outbreak (225). The typically low levels of genetic variation observed in *Campylobacter* outbreaks are similar to those observed among same-patient isolates (132), or following passage through a human or murine host (226-228).

2.31 In addition to using genomics to investigate suspected outbreaks, incorporating WGS into routine surveillance would improve our understanding of the long-term epidemiology of *Campylobacter* (229) and facilitate the accurate prediction of antimicrobial susceptibility profiles (83). Despite the growing application of WGS to problems in clinical microbiology (230, 231), by early 2017 it had not been used widely for surveillance of *Campylobacter*. This was largely because, even with decreasing sequencing costs, the volume of *Campylobacter* samples received made such a programme prohibitively expensive for most laboratories (232). The Campylobacteriosis in Oxfordshire project, funded by DEFRA and the FSA, has used WGS surveillance in near real time since 2011 (132), thereby demonstrating the feasibility of the approach for these organisms.

2.32 The results of the gene-by-gene analyses of 379 isolates from the 'Campylobacteriosis in Oxfordshire' project highlighted the value of WGS surveillance of *Campylobacter* (132). Overall, wgMLST identified a high degree of genetic diversity; although lineages differed with respect to the levels of variation present. This observation was supported by subsequent, smaller studies that reported heterogeneity in genetic variation among *Campylobacter* lineages and described certain clones that were stable over space and time (233, 234). Improving the understanding of the baseline diversity in *Campylobacter* populations is important in determining the lineage-specific similarity thresholds that are required to define 'outbreak strains' in epidemiological investigations (229). Routine WGS surveillance also provides an opportunity to detect diffuse or "cryptic" outbreaks, which are likely to be not detected against the high background of sporadic infections. WGS has been used to identify clusters of highly similar isolates that were temporally associated but otherwise not linked epidemiologically in the UK (132) and

to identify clusters of closely related *C. jejuni* isolates from different districts in Finland (233).

2.33 Although human disease isolates comprise the majority of *Campylobacter* genomes sequenced to date, the increasing availability of genomes of animal and environmental isolates facilitate tracing sources of sporadic infections at a higher level of resolution than MLST-based methods of attribution. Genetic models for source attribution based on WGS data remained in development at the time of writing, but genomic data had already been used to identify likely sources of infection. For instance, an analysis of 1,810 genes comprising the pan-genome of 884 *C. jejuni* genomes identified 15 novel host-specific genetic markers that were used to attribute French and UK clinical isolates to chicken and ruminants, detecting a possible geographic difference in the relative importance of these sources (235). Gene-by-gene comparisons linked Finnish human disease isolates to temporally related chicken abattoir isolates (236). The finding that *C. jejuni* from grey seals clustered with human disease isolates suggested either a shared source of infection (*via* agricultural contamination) or direct transmission (from human sewage), highlighting the potential role of human behaviour in spreading pathogens in the environment (237).

The role of WGS data in refining population and evolutionary analyses

2.34 The availability of WGS datasets from increasingly large ones has facilitated a number of comparative genomics and evolutionary studies, which have, for example: confirmed genome-wide introgression between *C. jejuni* and *C. coli* (154); investigated signals and mechanisms of host association and biogeography (235, 238-240) inferred recombination hotspots in *C. jejuni* (238, 241); revealed major structural differences among *C. jejuni*, *C. coli*, *C. lari*, and *C. upsaliensis* (242); provided insights into the biology and evolution of *C. lari* (243), *C. fetus* (244-247) and *C. iguaniorum* (248): and estimated the core genome of various subsets of *Campylobacter* species (249-253).

Application to functional studies

2.35 Historically, the field of functional genetics has been reliant on a ‘bottom-up’ approach, that is, researchers study the function of individual genes or operons. In

the genomic era, however, this type of study has been enhanced by the availability of WGS, as evidenced by insights obtained on the metabolism of *C. coli* and *C. jejuni* (63, 254). *Campylobacter spp*. are limited in their ability to catabolise carbohydrates, particularly glucose; but isotopologue profiling, in combination with WGS, showed that certain clinical and porcine *C. coli* isolates were able to metabolise glucose due to the presence of the Entner-Doudoroff (ED) pathway, which was located on a transferrable genomic island (254). Building on this work, it was found that 1.7% of 6,184 *C. jejuni and C. coli* genomes from diverse clinical, animal, and environmental sources contained the ED pathway (63). Further experimental work suggested that this pathway may promote biofilm formation and enhance stationary phase survival in some isolates.

2.36 The availability of WGS data has also facilitated 'top-down' functional studies, wherein statistical methods are used to identify genotypes underlying phenotypes of interest. First used to study the aetiology of human diseases, genome wide association studies (GWAS) have since been applied to a variety of microbes (255). The first bacterial GWAS detected factors underlying host-adaptation in *Campylobacter*: association mapping of 192 isolates showed that genes encoding vitamin B_5 biosynthesis were present in almost all isolates from cattle but were often missing in those from chicken (238). The diets of cattle and chicken are low and high in vitamin B_5, respectively; therefore, it was proposed that the genetic difference was due to adaptation to host diet. Subsequently, the same association mapping approach was used to study biofilm production in *C. jejuni*. A strong association was detected for genes involved in adhesion, capsule production, glycosylation, motility, and oxidative stress. However, differences in the genes involved in biofilm formation in the host generalist clonal complexes ST-21 and ST-45 suggested that this behaviour evolved independently in these lineages and may be important in the colonisation of multiple hosts (240). Most recently, association mapping has been applied to study survival of *Campylobacter* "from farm to fork", identifying factors over-represented in human disease isolates (53).

Outlook: future developments, opportunities and needs for* Campylobacter *genetics and genomics research

2.37 At the time of writing, there were ongoing and increasingly rapid

developments in genome analysis technology that were likely to be highly influential in bacteriology in general and the study of *Campylobacter* in particular. Whilst predictions about the development of technologies and their impact on understanding are inherently difficult, this section will outline some of these developments and their likely implications.

Technology developments

2.38 The NGS technologies that powered the large-scale application of genomic approaches to studies of *Campylobacter* generated short sequences (of the order of 10^2 bp), which could be either mapped onto existing reference sequences or assembled *de novo* into contigs. Both of these approaches generated large volumes of high-quality data for the relatively short (1.6 Mbp) and uncomplicated *C. jejuni* and *C. coli* genomes, which have comparatively few repeat regions that assemble poorly with short read sequences; however, these data have limitations in the analysis of genome rearrangement and extrachromosomal elements and other components of the accessory genome, such as plasmids, phage and insertion sequences, which can play important roles in *Campylobacter* biology. It is interesting to note that the genomes of other *Campylobacter* species, not covered in this report, can be more complex in their structure and diversity (242, 246-248, 256, 257).

2.39 The alternative to the reliance of NGS on generating very large numbers of short sequences is 'single molecule sequencing' approaches, which determine the sequences of very long (10^3-10^5 bp or more) single DNA molecules. These technologies have the advantage that they can sequence through regions of genomic complexity and the long reads assemble into complete genomes much more readily and reliably. They are especially useful for determining if repetitive genome elements are on episomal plasmids or phage or in the chromosome, for example; however, at the time of writing these methods exhibited relatively high inaccuracy rates in each read, requiring high redundancy to assure sequence accuracy. Two different technologies exemplify this approach: Pacific Biosciences (PacBio) Single Molecule Real Time (SMRT) sequencing (258) and Oxford Nanopore sequencing (259). Briefly, SMRT sequencing uses a laser to detect the incorporation of individual nucleotides at a single DNA polymerase molecule, whilst the Nanopore approach detects changes in electrical potential across a membrane

as single stranded DNA passes through a molecular pore (the nanopore). Both technologies achieve very long reads. SMRT sequencing requires an extremely expensive instrument and is relatively expensive per sample, but can generate high quality data, along with other epigenetic information, specifically on the methylation status of bases. Nanopore uses an extremely low-cost device (the MinION and the related GridION, PromethION and SmigdION), which can be attached to the USB port of a personal computer and is relatively inexpensive to run, but generates information of more variable quality. Other technology platforms are under development, and it is, of course, always possible that additional approaches will be developed. The near-term implications of these technologies are discussed below.

Real time and near-patient testing, including diagnosis

2.40 NGS, SMRT sequencing and nanopore sequencing all have potential application in diagnostic microbiology (260), although cost and speed remain an issue with these approaches. The nature of the PacBio technology combined with its cost make it likely to be more suitable for reference laboratory functions and research than near patient testing. Perhaps the most exciting prospects are illustrated by the Nanopore approach. The very low cost and high portability of these instruments, which have been used in the field in during Ebola and Zika virus outbreaks, have the greatest potential for point-of-care testing. In-clinic molecular testing has been shown to be possible with molecular platforms, such as the GeneXpert MTB/RIF assay for tuberculosis diagnosis and susceptibility testing, with considerable advantages for patient management (261). A rapid inexpensive testing device of this type would also be invaluable in the food and agriculture industry for the detection of *Campylobacter* in food, food animals and the farm environment, for example. One possible implication of the development of such tools would be a reduction in the culturing of isolates from clinical specimens or samples from animals or the environment, the consequences of which, in terms of not having isolates available, would have to be considered carefully. High-throughput sequencing has been used increasingly for metagenomics or community analysis. In this application, DNA is prepared from a sample (patient, food, animal or environmental) and the microorganisms present determined by sequence analysis. The conventional means of achieving this was the amplification and sequencing of the 16S rRNA genes

present (either by conventional or NGS approaches), to determine the relative abundance of different bacterial genera. Whilst a successful approach, this method lacks resolution. More recently NGS approaches have been applied to sequencing the entire DNA present in the sample, and it has been possible, for example to detect *Campylobacter* MLST alleles in such samples (262).

Conclusions

2.41 Nucleotide sequence analyses have enabled substantial advances to be made in the understanding of the biology of *C. jejuni* and *C. coli* over the preceding twenty years. Robust methodologies had been established, which enabled: (i) precise isolate characterisation; (ii) high-resolution outbreak investigations; (iii) the establishment of the population structure of *C. jejuni* and *C. coli*; (iv) investigations into *Campylobacter* evolution; and (v) improved understanding of the pathways of human infection though attribution analyses. In the immediate future, improved and even more cost-effective means of conducting these analyses can be anticipated, although it is likely that the most dramatic reductions of cost occurred in the 2000-2017. The development of high resolution near-patient characterisation, preferably from complex clinical specimens, remains a major goal which could be anticipated to be achieved in near future. Other than perhaps resolving multiple infections, this technology is unlikely to transform understanding of human infection. A technological development that has the potential for a major improvement in understanding, to be discussed elsewhere in this report, is the development of improved attribution methods on whole genome sequence data. Finally, in line with other foodborne pathogens (263), it is likely that cgMLST methods will become the international standard method for *Campylobacter* typing.

Recommendations

2.42 That sequence-based typing remains the basis for the characterisation of *Campylobacter jejuni* and *Campylobacter coli*.

2.43 Where practicable, sequence-based typing is best achieved using WGS data and the cgMLST analysis approach. When WGS is not practicable or

achievable, a combination of conventional MLST and single locus typing (*porA* and *fla* typing) approaches can be used.

2.44 Regard should be given to the possible impact of developments in:

- **(i) Nucleotide sequencing technologies that enable near patient and complex sample analysis;**
- **(ii) Improved attribution to source using WGS data.**

Tables and figures

Table 2.1. Specialist genome databases that contain *Campylobacter* WGS data

Database[a]	***Campylobacter* genomes (*n*)**[b]	**Total genomes (*n*)**[b]
Ensembl Bacteria	368	44,048
JGI Genome OnLine Database (GOLD)	4,955	249,377[c]
JGI Integrated Microbial Genomes and Microbiome Samples (IMG/M)	645	53,492[c]
Pathosystems Resource Integration Center (PATRIC)	2,394	104,126
Ribosomal Multilocus Sequence Typing (rMLST) PubMLST database	16,735	216,528
C. jejuni/*coli* PubMLST database	11,453	52,727[d]
Non-*jejuni*/*coli* PubMLST database	2	349[d]

[a] JGI, Joint Genome Institute

[b] As of July 2017

[c] Bacterial genomes only

[d] Total number of *Campylobacter* isolates

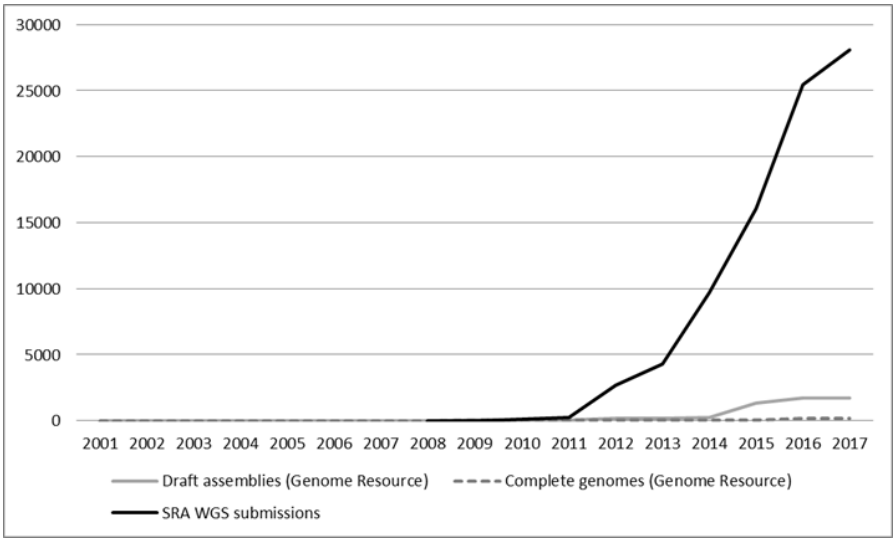


Figure 2.1 Availability of *Campylobacter* genomes in the NCBI Short Read Archive and Microbial Genomes Resource between 2001 and 2017.

Chapter 3: Epidemiology of *Campylobacter* infection in Humans

Introduction

3.1 Since it was first identified as a human pathogen in the 1970s *Campylobacter* has become a leading cause of acute gastroenteritis worldwide (264-266). The clinically relevant major pathogens are *C. jejuni* and *C. coli*, but several other species also cause illness in humans (267).

3.2 As well as causing acute, inflammatory enteritis *Campylobacter* infection may lead to serious chronic sequelae with substantial personal, healthcare and societal costs (268).

Public Health Impact

Illness burden

3.3 The population burden of illness associated with *Campylobacter* infection is very high. In the United Kingdom the burden of *Campylobacter* infection has been estimated in a population-based prospective cohort study and a prospective study of presentations to primary care (the Second Study of Infectious Intestinal Disease in the Community (IID2 study)). Around 500,000 people were found to be affected *Campylobacter* in a year (annual incidence = 10 cases per 1,000-person years (269, 270). As well as defining illness burden, a secondary objective of the IID2 Study was to re-calibrate national surveillance systems, i.e. to estimate the amount by which the number of laboratory-reported cases of infection with specified pathogens needs to be multiplied to establish the actual number of infections in the community. So, for every case of *Campylobacter* reported to national surveillance centres in the UK, approximately 9 cases had occurred in the community (269).

3.4 Turning to foodborne transmission, *Campylobacter* spp. are estimated to cause some 96 million (95% uncertainty interval (UI) 52 - 177 million) cases of foodborne illness worldwide (265). In the UK, there are an estimated 280,000 cases of *Campylobacter* foodborne illness annually (271) whilst in Canada the figure is around 145,000 cases (272).

3.5 An increasingly common way of describing the burden of disease is the disability-adjusted life year (DALY), which combines loss of life and health due to

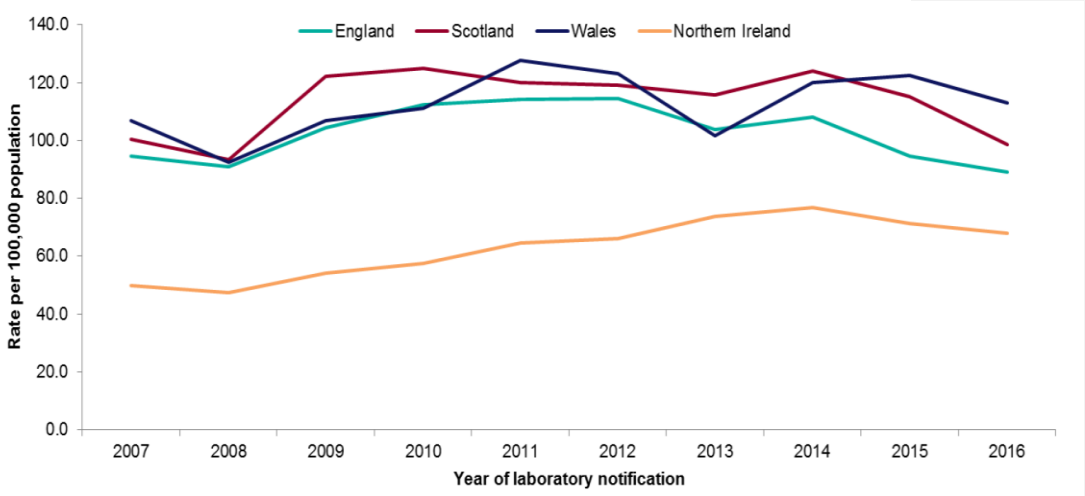
illness compared with a “perfect” state of health, using time as the common measure. In the US, *Campylobacter* infection is estimated to cause about 22,500 DALYs annually (273), whilst in the Netherlands *Campylobacter* spp. are responsible for around 3,600 DALYs per year (274). In a recently completed study in the UK the largest burden of microbiological foodborne disease was associated with *Campylobacter* (approximately 70,000 QALYs over a lifetime) (275).

Routine surveillance data

3.7 Figure 3.1 shows the annual reporting rate for laboratory-confirmed *Campylobacter* infection in the UK between 2007 and 2016. The reporting rate for *Campylobacter* decreased in the UK from 96.9 per 100,000 population in 2015 to 90.5 per 100,000 in 2016. In England reported *Campylobacter* infection rates declined in 2016 to the lowest rate reported since 2008 and remained below the rates observed in Wales and Scotland (Figure 3.1). Northern Ireland continued to report rates lower than the rest of the United Kingdom (67.9 cases per 100,000 population). All countries reported fewer cases in 2016 than in 2015 but the largest fall in reported rates occurred in Scotland. However, in 2017 (at the time of writing in week 47) laboratory-reported cases of *Campylobacter* in England and Wales had risen again from 48,835 in 2016 to 51,538 in 2017 (276). In Northern Ireland *Campylobacter* reports had risen from 377 at the end of quarter 3 in 2016 to 495 at the end of quarter 3 in 2017 (HSC Public Health 277). Data for 2017 in Scotland are not yet publicly available.

3.8 Up to 20% of *Campylobacter* cases report travel overseas during the incubation period (278). Just under half of the 17% of *Campylobacter* cases from North East Scotland who report foreign travel are infected with strains attributed to chicken (145).

Figure 3.1: Annual reported rates of laboratory-confirmed *Campylobacter*, UK, 2007-2016



Sources: Public Health England, Health Protection Scotland, Public Health Wales and Public Health Agency of Northern Ireland

3.9 Correcting for under ascertainment using the UK reporting ratios from the IID2 study the rate of campylobacteriosis in the UK population has hovered round 1,000 cases per 100,000 since 2009.

3.10 Routine surveillance remains key to understanding trends and **we recommend that the Food Standards Agency and its equivalents in the devolved administrations continue to work closely with their counterpart health protection organisations to maintain routine surveillance for gastrointestinal pathogens in general and *Campylobacter* in particular**.

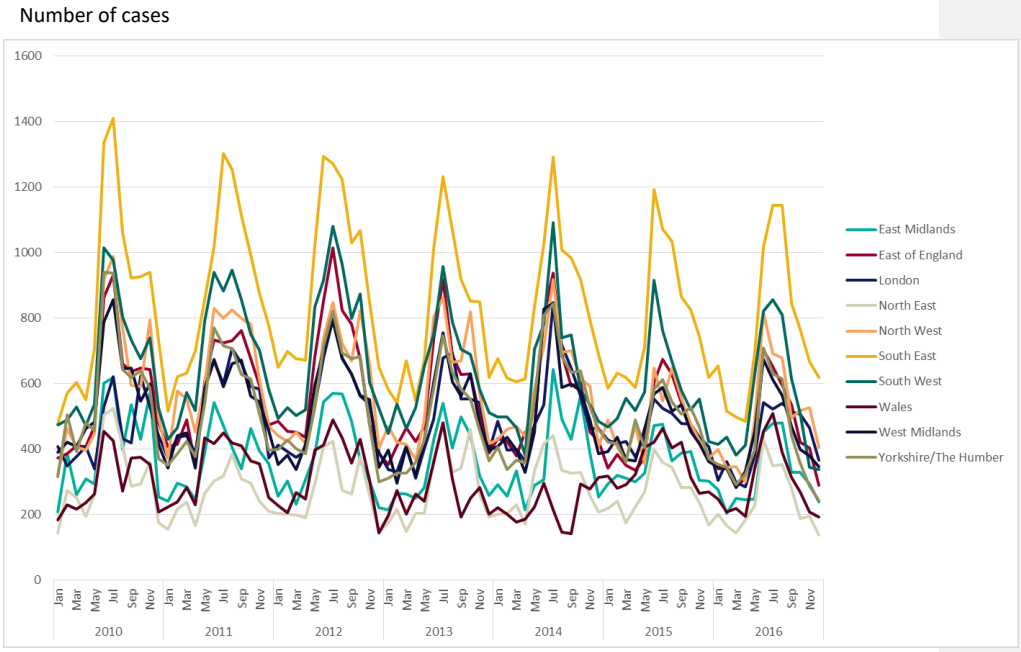
Dose Response

3.11 As few as 500 organisms of *Campylobacter* caused illness following a self feeding trial and this led to the general conclusion that the infective dose is fewer than 500 cells (279). Several dose response models have been developed for campylobacteriosis (280, 281). These are based on the single hit concept which states that one organism is sufficient to cause disease but the probability of that occurring may be very small. These studies used dose-response data from human outbreaks (282) and/or feeding studies on adult volunteers (283). The concept of the ID50 (the dose at which 50% of individuals challenged become infected) is a more meaningful concept than the infective dose because *de facto* for single hit models the infective dose is 1 organism. The ID50 from human feeding studies is approximately 900 cells (280, 281). More recently a dose response relationship was observed in an outbreak caused by chicken liver pâté where increasing the dose (amount of pâté eaten) corresponded to an increase in the risk of disease (284). Modelling work incorporating the effect of acquired immunity on the dose response has demonstrated pronounced effects on absolute and relative risk estimates for *Campylobacter* infection (285).

Seasonality

3.12 One of the defining features of *Campylobacter* infection is marked seasonality (Figure 3.2) (145, 286). In developed countries, human *Campylobacter* infection peaks in late spring (145, 287). To date, various hypotheses for the seasonal pattern observed include climate (287, 288), fly hatching (289), seasonal poultry contamination (290-293) and barbecuing (294). More likely is that interactions between several factors (food, environmental and social) produce this seasonal peak. This means that both natural science and social science methods are required to investigate transmission pathways within an interdisciplinary framework and then, subsequently, to assess and evaluate interventions.

Figure 3.2: Seasonal Pattern of laboratory-reported *Campylobacter* cases in England and Wales, 2010 to 2016



Source: Public Health England

Outbreaks

3.13 Table 3.3 summarises food-related risk factors in outbreaks of human *Campylobacter* infection identified in studies published in the peer-reviewed literature between 2006 and 2016.

3.14 The association between eating undercooked poultry and developing *Campylobacter* infection is well established. However, consuming lightly cooked chicken livers, chicken liver pâté and chicken liver parfait have emerged as important risk factors (224, 225, 284). Recognising this emerging trend in the UK, the Food Standards Agency commissioned research to develop a recipe for manufacturing commercial quantities of chicken liver pâté that reliably kills *Campylobacter* (295).

3.15 Further evidence that cooking practices are responsible for *Campylobacter* cases and outbreaks associated with lightly cooked chicken livers comes from an interdisciplinary study in the UK (296). In an online survey, most chefs who took part could correctly identify safely cooked chicken livers; however, they tended to overestimate consumers' preference for "pinkness" and so chefs tended to serve chicken livers more lightly cooked than the public would have preferred. Moreover, it was estimated that 19%-52% of livers served commercially in the UK do not reach the recommended cooking temperature of 70°C and that predicted *Campylobacter* survival rates in those undercooked livers were between 48% and 98% (296).

3.16 Consuming raw milk, either intentionally or through failure of pasteurisation, continues to pose risks for *Campylobacter* infection (220, 297). More than 50 people were affected with *Campylobacter* in November and December 2016 after consuming raw milk from a vending machine on a farm in Cumbria (298). In June 2017 raw milk was implicated in an outbreak of *Campylobacter* infection affecting four people in the South West of England (299). These recent events reinforce the importance of pasteurisation as a control for a range of pathogens, including *Campylobacter*, in milk. **It is important, therefore, that the FSA should continue to warn the public of the health dangers of raw milk (See also Chapter 6).**

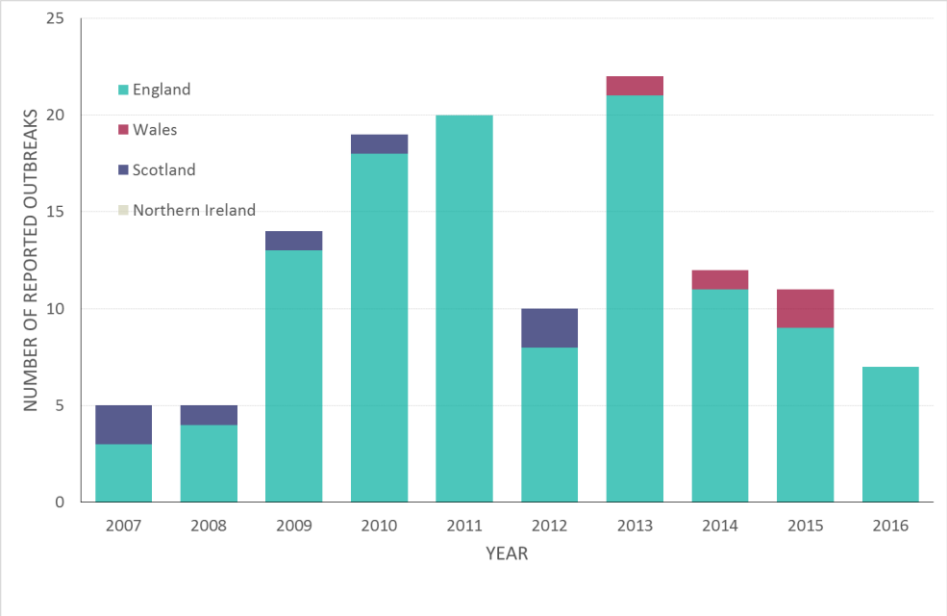
Outbreak surveillance data, UK

3.17 Outbreaks that have been investigated meticulously may provide useful data for apportioning diarrhoeal disease by transmission route; however, there are several potential limitations to interpreting the results. These include the robustness of evidence incriminating a food vehicle in an outbreak the first place (300) and an assumption that the distribution of food vehicles implicated in outbreaks is the same as the distribution of those responsible for sporadic cases of infection. Testing the latter assumption, Ebel (301) in the US conducted a comprehensive analysis of FoodNet data for a variety of foodborne pathogens, which, in the main, supported the acceptability of outbreak-based source attribution methods. Using outbreak surveillance data in this way helps to overcome publication bias (302).

3.18 Figure 3.3 shows the number of outbreaks reported to public health agencies in the UK between 2006 and 2016 (N=125). Most outbreaks were reported from

England (N=114) and none were reported from Northern Ireland. Reporting has declined since 2013 but it is unclear if this reduction is genuine or the result of a surveillance artefact.

Figure 3.3: Number of reported outbreaks of Campylobacter, UK, 2007-2016 (N=125)



Sources: Public Health England, Health Protection Scotland, Public Health Wales and Public Health Agency of Northern Ireland

3.19 It is worth noting that detection of small outbreaks and source attribution for individual cases of *Campylobacter*, whether sporadic or part of an outbreak, tends to be derived from questionnaire data from interviews conducted with individuals with microbiologically confirmed infection. Such microbiological confirmation usually relies on the affected individual making contact with healthcare services and submitting stool samples. Information is then obtained by the individual being interviewed in a timely fashion, often by Environmental Health professionals. Changes in interviewing practices, staff resources, case follow up rates, healthcare access, or changes in the population that submit stool samples may potentially have an impact on reported

rates of *Campylobacter* cases, number of reported outbreaks and epidemiological conclusions derived about source attribution of cases (286). Over recent years there is evidence that contact with primary care has changed, with an increase in telephone consultations and changes in access to healthcare professionals (303). In addition in one audit case in Wales, interview practices and follow up rates for *Campylobacter* cases varied greatly across different areas and have the potential to change again with implementation of new guidance or change in practice (304). Variations in data gathering methods and completeness of follow up of cases will have an impact on epidemiological conclusions drawn at a national level about risk factors for acquiring infection.

3.20 It is also worth noting that the Environmental Health Professional workforce is shrinking. For example, the number of full time equivalent staff employed by councils in Wales to deliver Environmental Health services fell by 16.4 per cent between 2011-12 and 2013-14 (305). In England the average budget for Environmental Health services, after taking account of inflation, fell in real terms by 6.8% between 2013-14 and 2015-15, and was expected to fall by a further 30% in 2015-16. Staffing cuts as a result of budgetary pressures were spread across most service areas but those most affected were environmental protection (including noise control) followed by food safety, and health and safety services. These staffing reductions have led to changes in the risk prioritisation of food and health and safety inspections to reduce service demands (306). In Scotland the number of Environmental Health Professionals fell by 11.5% between March 2009 and September 2012 and the number of Food Safety Officers fell by almost 21% over the same period (307). The reductions in the Environmental Health Professional workforce across the UK might well have had an impact on the ability to follow up individual cases of *Campylobacter*.

3.21 The total number of people affected in outbreaks of *Campylobacter* is shown in Table 3.1.

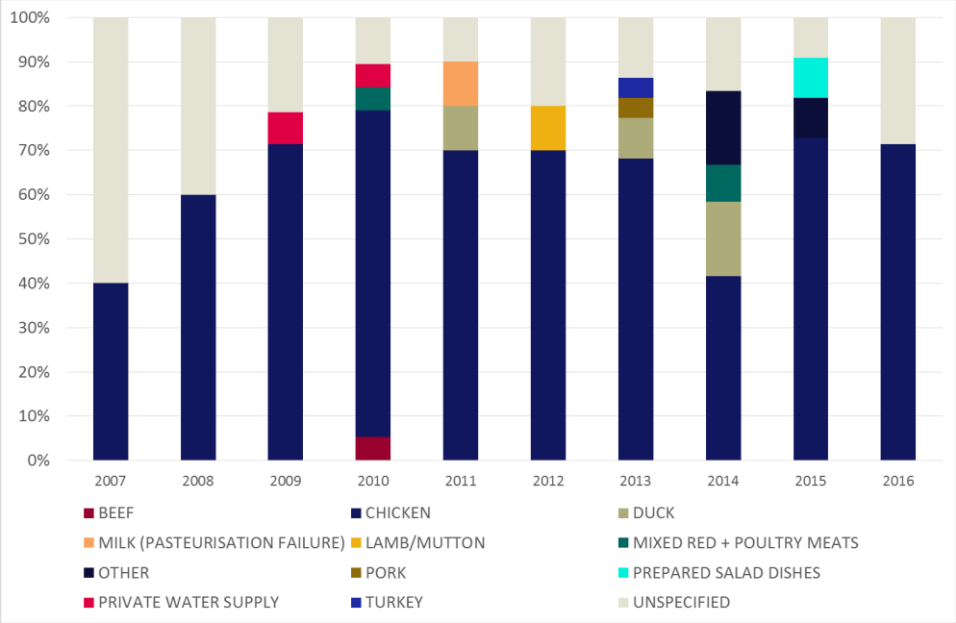
Table 3.1: Cases affected in *Campylobacter* outbreaks, UK, 2007-2016

	Year									
	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
Total affected	118	55	321	394	520	116	407	130	190	152
Min affected	7	3	2	2	4	2	3	2	4	3
Max affected	52	29	167	90	84	39	56	39	44	51
Mean affected	23.6	11	22.9	20.7	26.0	11.6	18.5	10.8	17.3	21.7
Median affected	12	10	15	18	22.5	6.5	19	10	14.5	15

Sources: Public Health England, Health Protection Scotland, Public Health Wales and Public Health Agency of Northern Ireland

3.22 The food vehicles implicated in *Campylobacter* outbreaks in the UK are summarised in Figure 3.4. Poultry predominates (N=83/125 outbreaks).

Figure 3.4: Food vehicles implicated in outbreaks of *Campylobacter*, UK, 2007-2016 (N = 125)



Sources: Public Health England, Health Protection Scotland, Public Health Wales and Public Health Agency of Northern Ireland

3.23 *Campylobacter* is also one of the most common causes of food-related illness in Europe (308). Using outbreak data, 29% of *Campylobacter* cases that could be allocated to a source were attributed to contaminated poultry. However, most cases could not be attributed to a source, illustrating another limitation of using outbreak data for attribution purposes. It should be noted that person to person transmission is very rare.

Sporadic infection

3.24 Most *Campylobacter* cases are unrelated to outbreaks. Table 3.4 contains a summary of food-related risk factors for sporadic human cases of *Campylobacter* infection identified in international studies published between 2006 and 2016. Eating contaminated poultry in one form or another continues to dominate the epidemiology of sporadic cases (309, 310).

3.25 Newly identified food-related risk factors for sporadic infection include contact with garden soil (*C. jejuni* and *C. coli*), consuming beef (*C. coli* only) (310), and eating cantaloupe and queso fresco (Mexican cheese) (311).

3.26 Perhaps the most persuasive evidence for the role of contaminated food commodities in causing *Campylobacter* infection comes from studies that describe the impact of interventions. In New Zealand, a range of voluntary and regulatory interventions were introduced in 2006 to tackle the very high incidence of *Campylobacter* infection by reducing contamination in poultry. Since then, there has been a 74% reduction in *Campylobacter* cases attributable to contaminated poultry, illustrating the success of a population-level food safety response to a serious public health threat (148). This reduction has persisted (149). Moreover, rates of Guillain-Barre Syndrome, a serious consequence of Campylobacter infection, have also declined (312).

3.27 In the UK the Food Standards Agency, Defra, the UK poultry industry and major retailers agreed target reductions in the levels of *Campylobacter* on chickens (See also Chapter 5). Based on reducing numbers of the most contaminated birds (>1,000 colony forming units (cfu) per gram) in UK poultry houses from 27% to 10% by 2015, it was estimated that there would be an accompanying reduction of *Campylobacter* cases of up to 30% (313). Data from a survey to examine levels on carcases have shown a decline in the number contaminated with *Campylobacter* and those with the highest levels of contamination (314). However, changes in survey methodology part way through make interpretations of trend problematic. Nevertheless, **we recommend that the Food Standards Agency and its equivalents in the devolved administration continue to monitor *Campylobacter* levels on carcases at retail sale.**

3.28 Natural experiments also emphasise the importance of poultry contamination as a major source of human *Campylobacter* infection. For example, in The Netherlands widespread culling of poultry took place because of avian influenza and there was a contemporaneous decline in human *Campylobacter* infection, especially in the culling areas (315).

3.29 Finally, non-food-related risk factors for *Campylobacter* infection include exposure to contaminated water (drinking water or recreational water)(316, 317), foreign travel, direct contact with farm animals (316, 318) and use of acid-suppressing medication (319) including proton pump inhibitors (PPI) (320, 321). However, in a recent retrospective cohort study of *Campylobacter* incidence following the first prescription of a PPI, people who were prescribed these medicines were found to have a greater underlying risk of gastrointestinal (GI) infection beforehand and to have a higher prevalence of risk factors before PPI prescription. The rate of diagnosis of infection increased with time regardless of PPI use, and there was no evidence that PPI was associated with an increase in diagnosed GI infection. The authors concluded that factors associated with the demographic profile of the patient were the main contributors to increased rates of GI infection for patients prescribed PPIs (322).

Clinical features and impact

Clinical features

3.30 Infections caused by *Campylobacter jejuni* and *C. coli* are indistinguishable clinically and most laboratories do not attempt to distinguish between the two organisms. Although *C. jejuni* is the predominant cause of campylobacteriosis, there are studies reporting 7% of isolates are *C. coli* in the UK and 18.6 % in Germany (323, 324).

Acute enteritis

3.31 Campylobacteriosis is usually characterised by an acute, self-limiting enterocolitis, lasting up to a week and clinical illness is often preceded by a prodrome of fever, headache, myalgia and malaise. Symptoms of disease often include abdominal pain, fever and cramps (frequently severe) and diarrhoea, which may be inflammatory, with slimy/bloody stools, or non-inflammatory, with watery stools and absence of blood. These clinical symptoms are often indistinguishable from GI infections caused by *Shigella*, *Salmonella* and *Yersinia* species. The incubation period is usually 2 to 5 days after ingestion, but may extend to 10 days (325). In recent analyses of outbreak data in Norway and New Zealand, the majority (>90% cases) have an incubation period of ≤ 5 days (326, 327). Although *C. jejuni* is

generally considered to cause a mild, self-limiting acute enteritis, in a recent study in Sweden over a quarter (27%) of laboratory-confirmed cases of *Campylobacter* were admitted to hospital (328). Most (92%) of the hospitalised were suffering from severe enteritis or colitis. People with co-morbidities were more likely to be admitted, as were people infected with *C. jejuni* ST-257. In the UK less than 1% of cases of campylobacteriosis are estimated to be hospitalised (271).

3.32 In many regions, where antibiotics are used in the empirical treatment of *Campylobacter* infections e.g. in elderly people, prolonged cases of enteritis, septicaemia or other extra-intestinal infections, some antibiotics such as fluoroquinolones are of limited use, due to antibiotic resistance. Erythromycin is still the drug of choice. There is some evidence of protective immunity after infection from volunteer studies and this may explain the higher incidence of disease in very young children. The efficacy of artificial immunogens is questionable since there is a wide variety of virulence between different phenotypes and an absence in increase of specific antibody. A small proportion (5-10%) of affected individuals suffer relapses, possibly caused by an incomplete immune response since immunocompromised hosts often have severe, extra-intestinal and prolonged illness.

Chronic sequelae

3.33 As well as causing very unpleasant acute symptoms, *Campylobacter* infection is also associated with various chronic sequelae although the evidence for an association is stronger for some conditions than others. *Campylobacter* infection has been implicated in the subsequent development of: reactive arthritis (ReA); Guillain-Barré syndrome (GBS) and Miller Fisher syndrome (MFS); haemolytic uraemic syndrome (HUS); inflammatory bowel disease (IBD); and functional gastrointestinal disorders (FGID) (268).

Reactive Arthritis

3.34 Reactive arthritis (ReA), previously known as Reiter's Syndrome, is a post-infectious spondylo-arthropathy, which occurs approximately two to four weeks after gastrointestinal or genitourinary infections. The weighted mean incidence of ReA following *Campylobacter* infection is estimated to be around 9 per 1,000 cases (329). Polymorphisms in the interleukin-18 and interferon-gamma genes appear to be associated with the development of *Campylobacter*-associated ReA (330).

3.35 Although symptoms usually disappear completely within six months, persistence beyond 6 months is seen in around 10–20% of people. Ongoing ReA arthritis beyond 12 months and requiring longer-term treatment is rare. Antibiotic treatment does not appear to improve the outcome in ReA (331).

Guillain-Barré Syndrome

3.36 Guillain-Barré syndrome (GBS) is the most severe late consequence of *Campylobacter* infection. It is an autoimmune peripheral neuropathy causing limb weakness that is sometimes fatal. Neural damage involves molecular mimicry between *C. jejuni* and human peripheral nerve proteins (332). It is known that sialylated lipo-oligosaccharides (LOS) of *C. jejuni* are essential virulence factors for the development of GBS; however, there is now a proposal that the polysaccharide capsule of *C. jejuni* is also a crucial virulence factor (Heikema *et al*, 2015). Engberg (333) concluded that 30-40 % of GBS cases are triggered by *Campylobacter* enteritis. This condition is thought to be associated with particular serotypes (e.g. O:19, O:4, O:5, O:41, O:2 and O:1). There are several pathological forms of GBS including demyelinating (acute inflammatory demyelinating polyneuropathy) and axonal (acute motel axonal neuropathy) forms. Host factors also play an important role in development of disease.

3.37 GBS presents with tingling in the toes, feet and legs, and the fingers, hands and arms. This is followed by ascending muscle weakness and paralysis (not to be confused with the descending paralysis of botulism). Symptoms can evolve very rapidly. By the third week after they first appear 90% of patients are at their weakest. Around 30% of patients with GBS have persisting weakness after 3 years and up to 3% can suffer a recurrence of muscle weakness and tingling sensations many years after the original episode.

3.38 GBS has become the most common cause of acute, flaccid paralysis since the eradication of polio, in most parts of the world and *Campylobacter* infection is now known as the single most identifiable antecedent infection associated with the development of GBS. The incidence of post-*Campylobacter* GBS is estimated to be between around 1 in 1,000 and 1 in 5,000 cases (334-336). The link to *Campylobacter* infection is strengthened by evidence from New Zealand, which showed that reducing human campylobacteriosis cases led to a contemporaneous

fall in the incidence of GBS (312). Often neglected features of GBS are fatigue, pain and psychological distress, which may have major effects on health-related quality of life(337).

Miller Fisher Syndrome

3.39 Miller Fisher syndrome (MFS) is a rare late consequence of *Campylobacter* infection. In effect, it is a non-paralytic variant of GBS in which patients present with ophthalmoplegia, ataxia and areflexia (338). The incidence is approximately one patient per one million population per year (339).

Haemolytic Uraemic Syndrome

3.40 *Campylobacter* infection is a rare cause of diarrhoea-related HUS and pulmonary-renal syndrome leading to life-threatening pulmonary haemorrhage (340).

Inflammatory Bowel Disease

3.41 There has been much debate over the years about a link between *Campylobacter* infection and inflammatory bowel disease (IBD). It has been hypothesised that in genetically susceptible people, gut microbes, in association with a disrupted gastrointestinal epithelium, can stimulate and then drive a dysregulated immune response leading to chronic inflammation in the intestine (341, 342).

3.42 In a recent systematic review and meta-analysis the association between IBD and various *Campylobacter* spp. was studied (343). An analysis stratified by species showed that the organisms principally accountable for an observed association with increased IBD risk were *C. concisus* (P-OR: 3.76, 95% CI 1.46 to 9.70, p value=0.006) and *C. showae* (P-OR: 2.39, 95% CI 1.11 - 5.18, p =0.027) (343).

Functional Gastrointestinal Disorders

3.43 The link between acute gastroenteritis and subsequent post-infectious irritable bowel syndrome (IBS) has been established for some time but there is a limited number of studies in which pathogen-specific risk has been quantified.

3.44 In a retrospective cohort study of FGID amongst the US military there were statistically significant associations between prior *Campylobacter* infection and the

risk of developing post-infectious IBS, functional dyspepsia, functional constipation and gastro-oesophageal reflux (344).

3.45 In a prospective study among active personnel enrolled in the US military's Millennium Cohort Study, risk factors for new-onset irritable bowel syndrome (IBS) included preceding acute gastroenteritis, female sex and anxiety syndrome. There was also a dose-response relationship with number of life stressors. Pre-existing anxiety or depression and acute gastroenteritis interacted with increased IBS risk compared with acute gastroenteritis alone (345). The complex interplay between intestinal microbiota and the autonomous nervous system (the so-called “gut-brain axis”) in conjunction with the immune system suggest that the gut-brain axis has a central function in perpetuating irritable bowel syndrome and that the intestinal microbiota play a crucial role (346).

Economic impact

3.46 A compelling way to capture the attention of politicians and policymakers is to attach a financial value to food-related illness. In high-income countries, diarrhoeal disease may be trivialised as inconvenient and unimportant alongside non-communicable diseases like diabetes, heart disease and stroke. Nevertheless, the disruption to society and the economy can be substantial (347).

3.47 Various researchers have monetised the cost of *Campylobacter* infection (Table 3.2). The estimates of cost vary quite widely reflecting differences in, for example, study design, costing elements included and type of healthcare system. Some researchers included in their cost estimates the impact of long-term sequelae whilst others did not. Despite the differences in study design the broad message is the same – namely that *Campylobacter* infection is expensive.

3.48 The likely costs of prevention can be hard to estimate but point to the fact that whilst the savings from prevention would accrue mainly to cases and health services the costs would lie elsewhere in government and in industry. Nevertheless in New Zealand, where there has been a considerable effort to reduce *Campylobacter* contamination of poultry flocks the benefit:cost ratio was extremely high (348). The beneficial effect of reduced campylobacteriosis to the New Zealand economy was

around NZD 57 million per year. Consequently, investing in food safety compliance measures at primary production seems to have been very worthwhile (348).

Table 3.2: Recently published estimates of the monetary costs of *Campylobacter* infection*

Year(s) of study	Country	Estimated cost per case	Estimated Annual Cost	Reference
2013	US	USD 2,283	USD 1.9 billion	(349)
2012-14	Switzerland	EUR 63–95	EUR 29–45 million	(350)
2011	Netherlands	EUR 706	EUR 76 million	(274)
2008-9	UK	GBP 85	GBP 50 million	(351)

* Data from O'Brien, 2017

Conclusions on what is known

3.49 *Campylobacter* remains the most common confirmed bacterial cause of acute gastroenteritis in the UK.

3.50 Routine surveillance remains key to understanding trends.

3.51 Contaminated poultry remains the greatest risk to humans but avoidable infections are also re-emerging in the UK associated with consuming raw (unpasteurised) milk.

Remaining uncertainties

3.52 It is not entirely clear at the time of writing that interventions in the food chain have yet led to a reduction in human disease.

Recommendations

3.53 We recommend that the Food Standards Agency and its equivalents in the devolved administrations continue to work closely with their counterpart Health

Protection organisations to maintain routine surveillance for gastrointestinal pathogens in general and *Campylobacter* in particular.

3.54 We recommend that the Food Standards Agency and its equivalents in the devolved administrations continue to monitor *Campylobacter* levels on chicken carcases at retail sale.

3.55 We recommend that the FSA should continue to warn the public of the health dangers of raw (unpasteurised) milk.

Table 3.3: Overview of food-related risk factors in outbreaks of human *Campylobacter* infection identified in studies published between 2006 and 2016

Year of publication	Year of outbreak	Country/Setting	Method(s)	Statistically significant food-related risk factors	Reference
2016	2012	Sweden	Microbiological using MLST and WGS	Eating chicken liver pâté	(224)
2016	2013	Australia/ University residential college	Retrospective cohort study (N = 56 cases/179 non-cases) and microbiological using wgMLST	Cohort study - Consuming chicken liver pâté entrée; Microbiological - *C. jejuni* and *C. coli* isolated in chicken liver pâté; wgMLST of clinical and food-derived *C. jejuni* isolates yielded three genetically distinct STs - ST528, ST535 (from clinical samples) and ST991 (from food samples).	(225)
2016	2014	US (Utah)	Microbiological	Drinking raw milk - three patient and one raw milk isolate yielding indistinguishable *C. jejuni* PFGE patterns.	(352)
2015	2014	US (Ohio & Oregon)/ Restaurant	Microbiological	Indistinguishable PFGE patterns from one clinical isolate and one chicken liver sample	(353)
2015	2011	UK (England)/ Community	Case-case analysis (N=37) and	Consuming milk where pasteurisation had failed	(220)

			microbiological using wgMLST		
2015	2007-12	US (26 States)	Outbreak surveillance (N = 81 outbreaks associated with raw milk)	The number of outbreaks of Campylobacter spp. associated with raw milk increased from 22 during 2007–2009 to 40 during 2010–2012.	(354)
2014	2012	UK (England)/ Catered wedding	Retrospective cohort study (N = 45 cases/32 non-cases)	Eating duck liver pâté	(355)
2014	2011	UK (England)/ Wedding reception	Retrospective cohort study (N = 49/non-cases = 48)	Eating chicken liver pâté clear dose–response between the quantity of chicken liver pâté	(284)
2014	2013	UK (England)/ Hotel lunch	Retrospective cohort study (N = 46 cases/92 non-cases)	Consuming roast turkey, consuming jus	(356)
2013	2011	UK/Catering college restaurant	Retrospective cohort study (N = 18 cases/14 non-cases) and microbiological using MLST	Cohort study – eating duck liver pâté Microbiological – using MLST isolates from duck samples were typical of isolates from farmed poultry.	(357)

2013	2005-11	Germany (Hesse)/ Community	Outbreak surveillance (N = 16 outbreaks with 5 or more cases)	Eight outbreaks probably associated with consumption of poultry Four outbreaks associated with the consumption of raw milk.	(358)
2013	2009	UK (north east England)/ Conference	Retrospective cohort study (N = 59 cases/48 non-cases	Consuming chicken liver pâté (Note this was a mixed outbreak – *Campylobacter* and *Salmonella* Typhimurium DT8).	(359)
2013	2012	The Netherlands	Case series (N = 3)	Drinking untreated milk	(360)
2013	2013	US (Pennsylvania)	Microbiological (N = 8 cases)	Drinking raw milk - *C. jejuni* isolates from patient stool (n = 1), bulk tank milk (n = 1), and retail milk (n = 1) were indistinguishable using PFGE.	(361) (362)
2013	2012	US (Multi-state)	Retrospective cohort study (N = 2 cases/40 non-cases) and Microbiological using MLST	Cohort study - eating charcuterie included a mousse made from chicken livers MLST – outbreak strain ST1212 found in six clinical isolates and one chicken liver isolate.	(363)
2013	2011	US (Alaska)		Consuming raw milk	(364)
2013	2012	US (Multi-state)	Descriptive epidemiology/ Microbiological using PFGE	Descriptive epidemiology 80/81 cases reported consuming raw milk PFGE - 2 unopened milk bottles yielded *C. jejuni* with an indistinguishable PFGE pattern to all clinical isolates	(365)

2013	2006 & 2007	US (California)/ 11 State correctional facilities & visitors to a dairy farm	Outbreak investigation (N = 1644 prisoners and 11 consumers) and Microbiological using PFGE and MLST	Microbiological - Environmental strains from two dairy farms implicated produced *C. jejuni* strains indistinguishable from the clinical outbreak strains.	(366)
2012	2012	Australia/ Birthday party at restaurant	Retrospective cohort study (N = 15 cases/42 non-cases)	Consuming chicken liver pâté	(367)
2012	2011	UK (England)/ Restaurant	Retrospective cohort study (N = 11 cases/15 non-cases) and Environmental Health investigation	Cohort study - consuming chilli sauce Environmental investigation pinpointed cross-contamination from raw chicken livers	(368)
2012		Spain /School	Outbreak investigation (N = 75 cases)	Eating roast chicken, eating Russian salad	(369)
2011	2008	US (Alaska)/ Community	Case-control study (N = 45 cases/90 matched controls) and microbiological	Eating raw peas contaminated by wild birds	(370) (371)

			using PFGE and then MLST		
2011	2001-8	Australia/Long-term care facilities	Outbreak surveillance (N = 8 *Campylobacter* outbreaks)	Suspected chicken consumption in one of eight outbreaks	(372)
2011	1992-2008	UK (England and Wales)	Outbreak surveillance (N = 79 *Campylobacter* outbreaks)	30 outbreaks associated with consuming poultry meat, 13 with miscellaneous foods*, 8 with milk/milk products, 6 with salad vegetables, 2 with sauces, 1 with fish/shellfish	(373)
2010	2010	UK/Wedding reception	Retrospective cohort study (N = 24 cases/36 non-cases)	Eating chicken liver parfait	(374)
2010	2009	South Korea/ School	Retrospective cohort study (N = 92 cases/199 non-cases)	Consuming chicken soup	(375)
2010	2005	Australia/ Boarding School	Retrospective cohort study (N = 35 cases/23 non-cases) and Microbiological using *fla*A-typing and MLST	Cohort study – eating evening meal (no single food item implicated) Microbiology - Two distinct MLST types (ST-49 or ST-52) and two distinct flaA-types (Fla 14b or 128) occurred in epidemiologically linked cases. Cross-contamination from chicken inferred.	(376)

2009	2005	The Netherlands/ School trip to a dairy farm	Case series and Microbiological using PFGE and *fla*A-typing	Case series - 19 of 22 symptomatic children tasted raw milk Microbiology - Human isolates and *C. jejuni* isolates from one of three samples of cattle faeces indistinguishable on PFGE and *fla*A typing.	(377)
2009	2007	The Netherlands/ Lunch at a dairy farm	Cohort study and Microbiological using PFGE and *fla*A-typing	Cohort study - Tasting raw milk Microbiology - *C. jejuni* from clinical isolates and a bulk milk sample indistinguishable using *fla*A-typing.	(377)
2009	2007	US (Kansas)/ Community fair	Retrospective cohort study (N = 68 cases/62 non-cases)	Eating fresh cheese made with raw milk	(378)
2009	2007	Austria	Outbreak surveillance (N = 104 *Campylobacter* outbreaks)	Food vehicles not stratified by pathogen in this paper	(379)
2009	2006	UK (Scotland)/ Restaurant	Retrospective cohort study (N = 7 cases/22 non-cases)	Eating chicken liver pâté	(380)
2009	2005	UK (Scotland)/ Farmers' dance	Retrospective cohort study (N =	Eating chicken liver pâté	(381)

			86 cases/78 non-cases)		
2008	2007	Sweden/ Catered buffet	Retrospective cohort study (N = 22 cases/35 non-cases	Eating marinated chicken	(382)
2008	Not stated	Austria/ Hospital	Case series (N = 7 patients & 14 staff)	Consuming poultry dishes prepared by the hospital kitchen (18 out of 21 cases)	(383)
2007	2005	Austria	Outbreak surveillance (N = 128 *Campylobacter* outbreaks)	Food vehicles not stratified by pathogen in this paper	(384)
2006	2004	Australia/ Restaurant	Retrospective cohort study (N = 11 cases/16 non-cases	Eating warm chicken salad, eating chicken mushroom (pollo funghi) pasta	(385)
2006	2005	Japan/ School	Epidemiological investigation (N = 36 cases/73 non-cases)	Attending cooking practice classes, handling raw chicken, eating cooked chicken	(386)
2006	2005	Denmark/ Canteen serving several companies	Retrospective cohort study (N = 79/non-cases = 168)	Consuming chicken salad	(387)

2006	2002-3	Finland/ Farming family	Case series (N = 6) and Microbiological using PFGE	Case series - All cases drank raw milk Microbiology – Indistinguishable *C. jejuni* PFGE profiles from clinical isolates, bovine faeces and bulk tank milk samples	(388)

Footnote: * Miscellaneous food includes buffet foods, sandwiches and other dishes comprising multiple ingredients

Table 3.4: Overview of food-related risk factors for sporadic human cases of *Campylobacter* infection identified in studies published between 2006 and 2016

Year of publication	Year of study	Country/Setting	Method(s)	Statistically significant food-related risk factors	Reference
2016	2010-13	Luxembourg/ Community	Case-control study (N = 548 cases/764 controls); Source attribution using MLST (N = 282 STs from 1,289 clinical isolates)	Risk factor analysis for *C. jejuni* and *C. coli* Chicken consumed at home; chicken consumed outside the home; poultry meat other than chicken consumed outside the home Risk factor analysis *C. coli* only Beef consumed at home; beef consumed outside the home; hamburger consumed outside the home Source attribution 61% poultry; 33% ruminant; 4.9% environmental water; <1% swine	(310)
2016	2010	US (Arizona)/ Community	Case-control study (N = 110 cases/61 Controls)	Eating cantaloupe; eating queso fresco (Mexican soft cheese); handling raw poultry	(311)
2016	2009-10	Israel/	Case-control study	Eating chicken	(389)

		Community (children <5 years only)	(N = 113 cases/113 matched controls)		
2015	2010-11	Norway/ Community	Case-control study (N = 995 cases/1501 controls)	Drinking purchased bottled water; eating chicken; eating undercooked meat; eating barbecued food	(294)
2015	2011-12	Tanzania	Case-control study (N = 136 cases/1059 Controls	Consuming chicken meat; consuming pre-prepared salad	(390)
2014	2012-13	Switzerland/ Community	Case-control study (N=159 cases/280 controls	Eating meat fondue (in particular chicken meat)	(391)
2013	2005-6	Scotland/ Community	Source attribution using MLST for *C. coli* only	Putative source for 40% of cases was chicken, with 60% acquired from other sources (ruminants 54% and pigs 6%)	(392)
2013		Denmark		Assessing the relative risk of becoming ill following exposure to Campylobacter on conventional or organic broiler meat indicated that the risk per serving from organic carcasses was 1.7 times higher than that of conventional carcasses	(392)

2012	2002-3	The Netherlands/ Community	Case-control study (N = 737 cases/3119 controls) combined with source attribution using MLST	Putative sources from source attribution were chicken (66.2%), cattle (20.7%), environment (10.1%), sheep (2.5%), and pigs (0.3%). For cases infected with poultry-associated strains the food-related risk factors was consuming chicken; For cases infected with ruminant-associated strains food-related risk factors were barbecuing (in rural areas) and consuming tripe; For cases infected with environment-associated STs the food-related risk factor was consuming game	(134)
2010		Greece (Attica)/ Community (children <15 years only)	Case-control study (N = 205 cases/205 matched controls)	Consuming chicken in the week prior to symptom onset	(393)
2010	2002-3	The Netherlands/ Community	Case-control study (N = 1315 *C. jejuni* cases/121 *C. coli* cases/3409 frequency-matched controls	*C. jejuni* – eating chicken *C. coli* – eating game and tripe Both – eating undercooked meat and barbecued meat	(320)
2010	2005-6	Spain (north east)/ Community	Case-control study (N = 81 cases/81 matched controls)	Consuming chicken three or more times in the seven days prior to symptom onset; consuming sliced deli meat handled “unhygienically” at retail stores	(394)

2009	2005-6	UK/ Community	Case-control study (N = 1592 cases/3983 frequency-matched controls	Regularly consuming chicken (>5 times a week); consuming commercially prepared chicken in the 5 days prior to symptom onset.	(319)
2009	2003-5	US (Washington State)/ Community (children <19 years of age)	Case-control study (N = 151 cases/580 matched controls	Consuming food from restaurants; suboptimal kitchen hygiene after preparing raw meat or chicken	(395)
2009	2003-4	Ireland/ Community	Case-control study (N = 197 cases/296 frequency-matched controls)	Consuming chicken; consuming lettuce; eating food from takeaway restaurants	(396)
2008	1999-2001	Australia (Hunter Valley)/ Community	Case-control study (N = 354 cases/593 unmatched controls)	All cases - household exposure to diarrhoeal illness, consuming restaurant chicken or beef, eating two or more ‘‘fast’’ food meals in a week, and travelling overseas Older cases only - eating restaurant-prepared red meat and Swimming	(316)
2008	2001-2	Australia/	Case-control study	Eating undercooked chicken; eating offal	(397)

		Community	(N = 881 cases/883 frequency-matched controls)		
2008	2002-4	France/ Community	Case-control study (N = 285 cases/285 matched controls)	Eating undercooked beef; eating at a restaurant; poor kitchen hygiene	(398)
2007	2002-4	US (8 FoodNet sites)/ Community (children <1 year of age)	Case-control study (N = 123 cases/928 controls)	Riding in a shopping cart (trolley) next to meat or poultry	(399)
2007	2001-2	Australia/ Community	Case-control study (N = 881 cases/833 controls)	Eating undercooked chicken; eating offal	(400)
2006	2000-1	Denmark/ Community	Case-control study (N = 74 cases/114 controls	Eating fresh chicken	(401)

Chapter 4: Source attribution of human campylobacteriosis

Introduction

4.1 Source attribution has been defined by Pires and colleagues (402) as: "…the partitioning of the human disease burden of one or more foodborne infections to specific source, where the term source includes animal reservoirs and vehicles (e.g. foods)." Attribution can be carried out at different points along the food chain (e.g. at the reservoir and at the food factory) (402). There are many approaches including expert elicitation, evaluation of the impact of interventions (or natural experiments), observational epidemiology (e.g. analytical epidemiological studies of risk factors) and analyses based on microbiological characterisation (typing or genetic analyses) allowing study of different aspects of the sources and transmission routes of this infection summarised in Figure 4.1. This chapter reviews the use of microbiological characterisation, and specifically analyses of *Campylobacter* genetic information to identify sources of human infection. The use of multilocus sequence type (MLST) genetic data in attribution analysis was recommended in the last AMCSF report on *Campylobacter* and has been widely applied since then. Approaches using these data are therefore covered in greatest detail. This genetic attribution review complements the epidemiology chapter that describes other evidence for sources and routes of infection, and the *Campylobacter* genetics and genomics chapter that reviews population genetic and genomic analysis of *Campylobacter* more generally.

4.2 Most work to determine *Campylobacter* sources since the last report has used either observational epidemiology or microbiological characterisation, especially the analysis of MLST data reviewed here. Analysis of MLST has focused on more upstream reservoirs and sources. Epidemiological studies are most effective in identifying the part of the transmission route close to the infected person (e.g. the contaminated food that was consumed). A highly simplified model of circulation of *Campylobacter* among animal reservoir sources leading to food contamination and disease is shown in Figure 4.2. This indicates our understanding that there is partial separation of the populations of *Campylobacter* among host species reservoirs but also some transmission across them. Transmission across reservoirs is more substantial among food animal species (403) than among some wild animals (404) and not precisely quantified for any. Accuracy of attribution to relatively isolated source

populations of *Campylobacter* such as those in various species of wild birds can be relatively accurate (405, 406) while there is less accuracy and certainty in attributing back to individual food animal species, where there is more transmission across host species boundaries. Examples of this are transmission between cattle and sheep which carry substantially similar populations of *Campylobacter* species, and even between these host species and commercial broilers (150, 239).

4.3 These limitations in accuracy and the associated potential for biased attribution of human disease to sources mean that analyses should be treated with appropriate caution, consideration given to validation using isolates from known sources with adjustment for biases observed, and results viewed in the context of other evidence such as that coming from observational epidemiology. Despite these limitations, the population genetic analysis of MLST has fulfilled the promise identified in the second ACMSF report and become the dominant approach to source attribution work for *Campylobacter*. It has proved useful in identifying the main sources of human infection with many studies using these approaches (Table 4.1). As well as allowing attribution to animal species reservoirs it has been possible to attribute to particular animal production systems, such as vertically integrated poultry production systems where individual producers may form the main reservoir for distinctive lineages of *Campylobacter* (150, 407).

4.4 While most applications to human disease surveillance have focussed on attributing disease burden to source the approach has also been applied to monitor specific industry interventions such as in New Zealand (140). In monitoring change over time, including for the evaluation of interventions, biases may be less important so long as they are consistent. With these possible uses in mind we review progress on source attribution in the context of the second ACMSF report, describe the methods that have been applied, summarise the findings from these studies, and consider limitations of the approach. We then outline future opportunities, challenges and directions for this approach, and the integration of this approach with other methods and data including the role of source attribution to study natural experiments and monitor interventions.

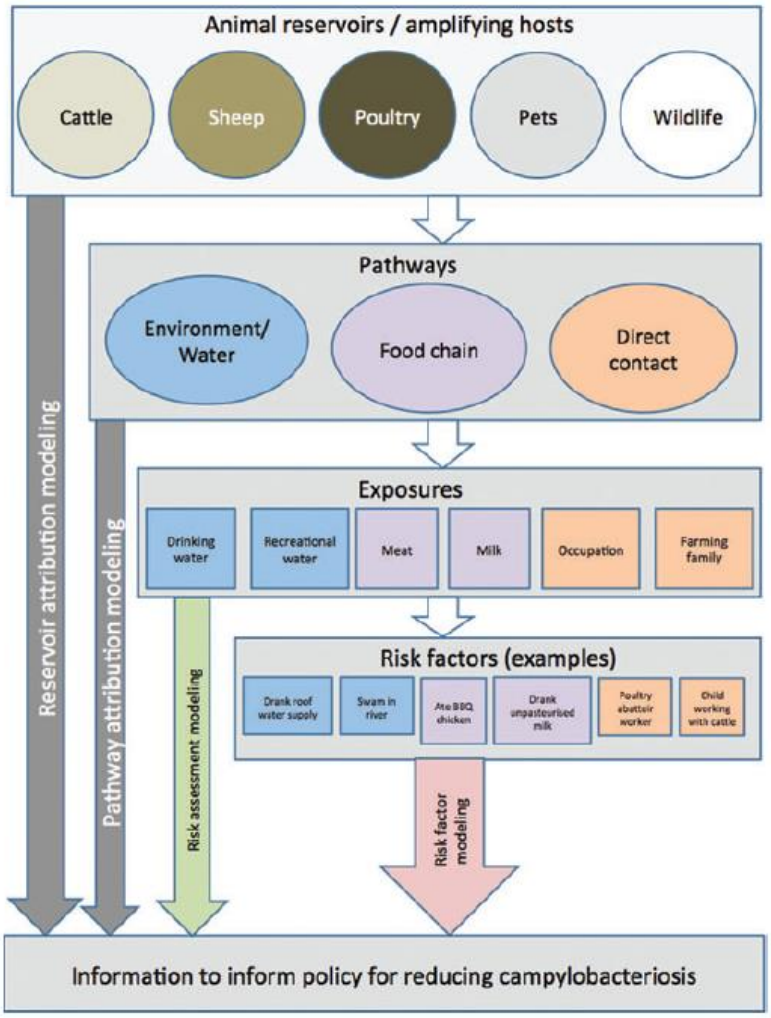


Figure 4.1. Schematic of attribution, risk assessment and epidemiological risk factor approaches for generating information to inform policy for reducing campylobacteriosis. Modified from (408).

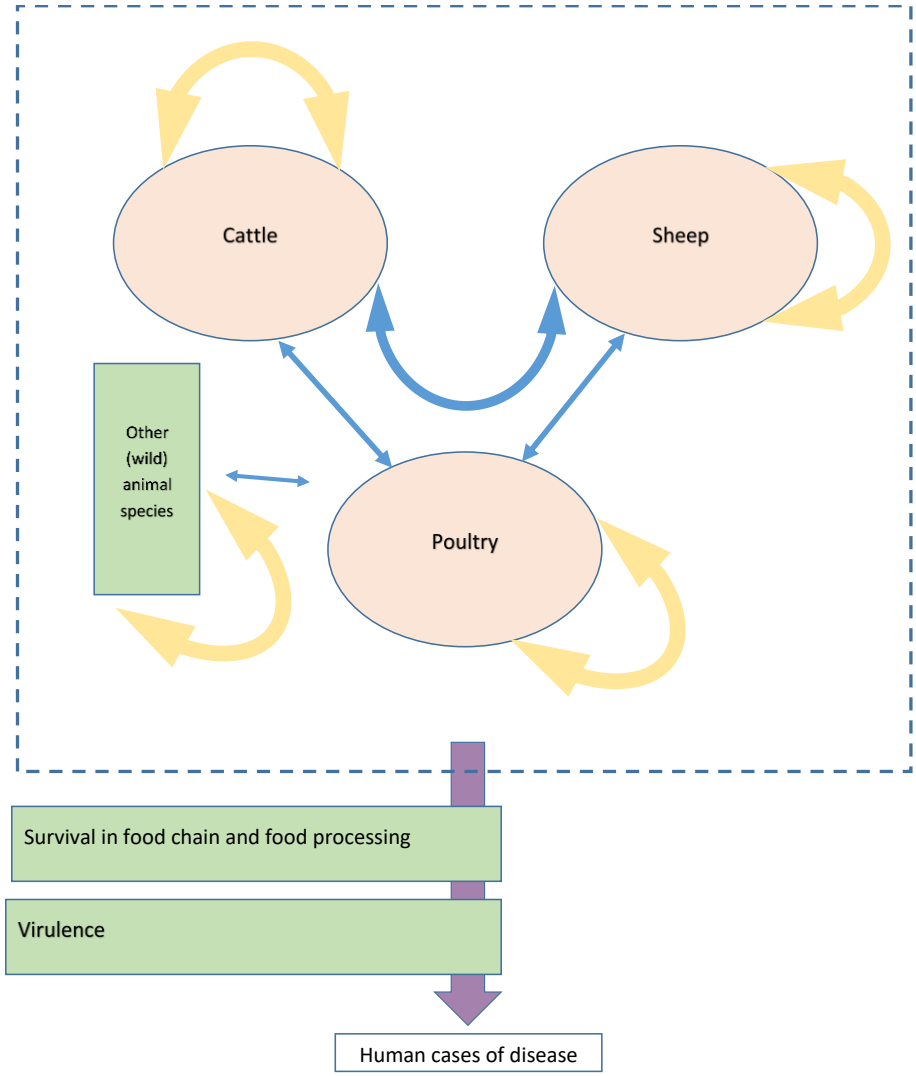


Figure 4.2. Within species circulation (yellow arrows) is generally higher than between species transmission (blue arrows). Between species transmission is higher among food animals than others. This as well as potentially varying virulence and survival in the food chain creates uncertainty quantitative estimates of the inferred sources of human infections.

Approaches to source attribution

4.5 Genetic source attribution studies require that (i) human infections arise from a range of sources and (ii) microbial subpopulations differ among these sources. The method requires both the actual separation among populations in different sources and an ability to estimate this from genomic data. The Second ACMSF report on *Campylobacter* identified that the "variability and genetic instability of *Campylobacter* cautions us against believing that there exists some magical solution to the typing of all *Campylobacters* of human significance*".* However, it was also noted that DNA sequence-based methods such as MLST and utilising whole genome sequencing offered the potential to help unravel the complex epidemiology of *Campylobacter*. In the 11 years since the report both the caution expressed and the predicted promise of pathogen genome sequencing have been confirmed. Approaches based on genetic sequence data have removed the concerns of type-ability and reproducibility discussed in method comparisons, which included methods other than direct sequencing, in the second ACMSF report.

Source attribution undertaken using sequence data

4.6 Seven locus MLST (121) has been established as the primary microbial characterisation approach for *Campylobacter* source attribution and has supported population genetic analyses markedly increasing our understanding of the sources of human infection and relationships between them. This work has also identified that overlapping populations of *Campylobacter* occur in different food animal species. This, due to transmission between different source animal species (Figure 4.2), contributes to the limitations of accuracy and certainty of source attribution from analysis of MLST data.

4.7 The discussion of analytical methods used below relates to experience with MLST data, which have been the dominant form of data analysed. These and similar methods could be used with different and more extensive genetic sequence data such as from whole genome sequencing and many lessons learned will apply to how best to use these more extensive data. A methodological review of source attribution utilising mathematical models was published in 2014 (409). Here we summarise the main approaches used with *Campylobacter* 7-locus MLST data briefly and their results (Table 4.1). Annex A provides technical details of the source attribution methods.

The Dutch Model

4.8 The Dutch model (409) is the simplest way to estimate the attribution of a particular genotype (e.g. ST) to a source, when the frequency distribution of each type is known for each source. It does this in terms of simple proportions. If the sequence type has a higher frequency in a particular source then it is more likely to be attributed to that source. The Dutch model can also be applied where there are multiple loci. Here, each locus is treated independently and then combined.

Hald Model and Modified Hald Model

4.9 This model was developed in Denmark for the attribution of human salmonellosis (410). This "Danish *Salmonella* source attribution" model uses a Bayesian framework with Markov chain Monte Carlo simulation to attribute sporadic laboratory-confirmed human *Salmonella* infections caused by different subtypes as a function of the prevalence of these subtypes in animal and food sources and the amount of each food source consumed. The model takes into account the uncertainty for all these factors and also includes travel as a possible risk factor.

4.10 This model was improved (140) to include the introduction of uncertainty in the estimates of source prevalence and an improved strategy for identifiability and is called the "Modified Hald Model" (141). This model does not include information on amount of food consumed.

4.11 In summary, the modified Hald model achieves source attribution by comparing the frequencies of human infections caused by different pathogenic subtypes (e.g. serotypes for *Salmonella* (140)), with the subtype frequencies found in the different sources accounting for potential subtype- and source-dependent characteristics, that may influence their chance to cause human illness (410)). This model is only implemented at ST level and has not been developed for multiple *loci*.

Population STRUCTURE

4.12 STRUCTURE is a Bayesian clustering model designed to infer population structure and to attribute individuals to population groups (137). The programme has

been used extensively with 7 locus *Campylobacter* MLST genotyping data [Table]. Each isolate is attributed on the basis of a training dataset consisting of isolates from known populations. The algorithm calculates the frequency of each particular sequence type in each population, treating each locus independently. Based on these frequencies, the probability of an isolate (e.g. a human isolate) belonging to a population group (e.g. sources such as bovine, ovine, poultry, swine etc.) is calculated. The programme allows users to select the number of steps in the Markov chain Monte Carlo process, how it is sampled, the length of burn in, and parameter values to reflect the user's population genetic assumptions of the relationships between populations.

Asymmetric Island Model

4.13 This source attribution model incorporates a Bayesian approach and uses the allelic profile of the sequence subtypes to reconstruct the genealogical history of the isolates (139). The host populations are considered to exist on separate "islands" (e.g. the sheep island). Mutations and recombination occur on each island. Migrations between each reservoir (island) into the human population are used to estimate the degree of attribution to each source and updated as the programme runs. This model has been extensively applied to *Campylobacter* 7 locus MLST data [Table].

Typological and non-typological approaches

4.14 Evaluation of approaches that summarise detailed data to a single type and compare types across studies have been shown to be less efficient in correctly attributing rare types (150). Extremely detailed sampling of source populations would be required to represent the diverse populations in each source allowing information to attribute each human isolate to source. Moreover, as well as being less efficient these methods summarising genetic data for each isolate to a type is likely to be less accurate even with extensively sampled populations. Specifically, if the biological process of recombination creates information that can allow prediction of source then analysis that captures this can be more accurate (150). Approaches that allow consideration of specific characteristics of each genetic sequence rather than summarising to type, such as the population genetic algorithms STRUCTURE and Asymmetric Island are therefore most commonly applied and considered more accurate using the genetic information available. STRUCTURE assumes

independence among the genetic loci analysed while the asymmetric Island algorithm models dependence between *loci*. This may produce greater accuracy and more statistically reliable estimates of uncertainty; however, neither STRUCTURE nor Asymmetric Island models allow integration of other information such as differential survival in the food chain, virulence, and other characteristics that come into play when attributing cases of human infection (Figure 4.2). The modified-Hald model has capacity to adjust for these features (141) and has been used by those wanting to consider other dimensions of data alongside genetic data (Table).

Validation and potential bias and inaccuracy

Self-attribution

4.15 Self-attribution is a key performance measure for source attribution models (142, 150). This is the average percentage accuracy with which any given isolate from a source can be correctly attributed back to its own source reservoir (e.g. the likelihood that the attribution model will assign an ovine isolate back to the ovine reservoir). Testing this can be performed in at least three ways: (i) the jack knife method, which splits the source datasets in two halves using the first half to parameterise the source attribution model; (ii) by carrying out the attribution using all of the source isolates and then re-introducing them blind to the models and determining their scores back to each source; or (iii) by using almost all reference data but testing one or a small number that are withheld from the sources and repeating this procedure. This type of validation is the most common and has allowed the demonstration of the extent to which populations, as indexed by 7-locus MLST, overlap between farm animal species. Most published studies reviewed did not publish validation results for their attribution method and dataset [Table]. Where this validation of accuracy attributing to reservoir was reported, bias identified was not adjusted for in attribution of human isolates [Table].

Population based source attribution results

4.16 Population based source attribution studies using genetic data, methods, and results are summarised in the table. These mainly attribute among food animal and other animal or environmental sources, although some studies considered attribution to travel and domestic sources. Generally, a large proportion of human case isolates are attributed to chicken, a small proportion to environmental sources, and an

intermediate proportion to other food animal reservoirs. A summary of the results on the proportion attributable to chicken sources across different analytical approaches is given in Figure 4.3. Asymmetric Island model analyses show a higher proportion attributable to chicken although the difference between this and STRUCTURE, for which comparison there is most data, does not reach statistical significance (p =0.0672). Two studies reported direct comparisons of models for attributing *C. jejuni* with Boysen et al comparing asymmetric island (67% attribution to chicken) with the a modified Hald model (52%) (146) and Sheppard et al Asymmetric Island (78%) and STRUCTURE (58%) (142). This study reports validation by self-attribution with asymmetric island (97% accuracy) performing better than STRUCTURE (70%). This empirical testing (STRUCTURE versus Asymmetric Island), and theoretical reasons outlined above to expect higher accuracy for the Asymmetric Island over a Hald model suggest that the Asymmetric Island results in these two studies may be the more accurate. In each Asymmetric Island estimated a higher proportion as coming from a chicken source than the comparator method. The geographical location of reference compared to human isolates may also affect results and accuracy (235, 411) as may the sample sizes (411). Given these differences the relative consistency in the main results across studies performed in different countries and using different reference datasets and methods is striking. All studies attribute more cases of human infection to chicken than to any other source and all show that there are important other sources in addition. In many studies the reference datasets for chicken are substantially larger than those from other possible sources which is a potential source of bias. The relationship between the excess percentage of isolates attributed to chicken and imbalance in the reference dataset is summarised in Figure 4.4. This comparison does not provide evidence for this excess of chicken isolates in some reference datasets creating a substantial bias although more formal testing is warranted.

4.17 In summary, chicken was the dominant but not the only source across all studies, accounting for an estimated 43%-87% of human disease in all populations. There is substantial scope for methodological refinement but no reason to expect that this main conclusion is due to problems with the method.

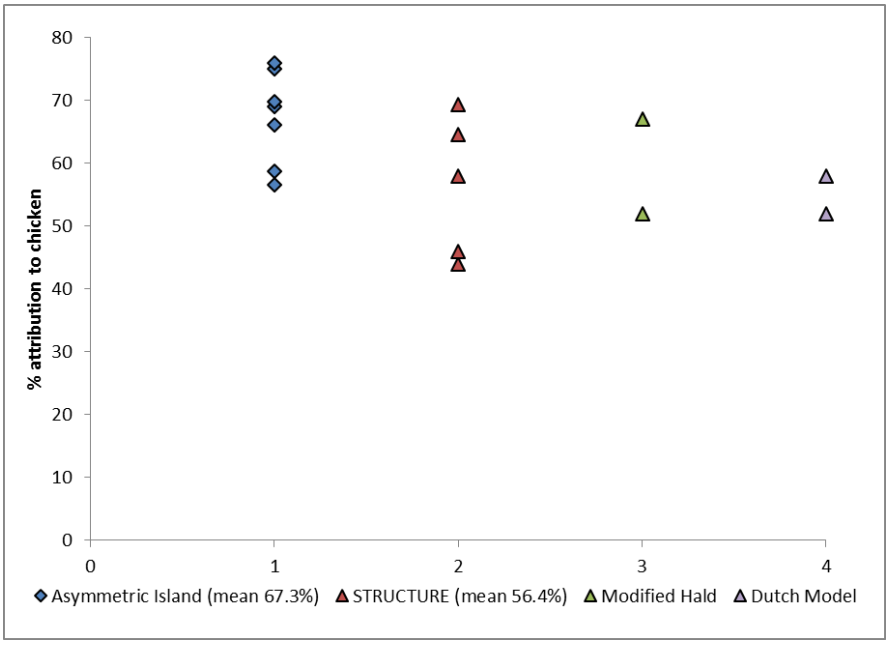


Figure 4.3. Proportion of clinical *C. jejuni* isolates attributed to chicken by studies using different models.

Table 4.1. Summary of published studies using multilocus sequence typing data (MLST) for source attribution, arranged by model.

Attribution Model	Source Animal Dataset	Clinical Dataset	Species	Attribution to Sources (%)	Self-attribution	Other	Comments	Reference
Asymmetric Island	Chicken 400 Cattle 168 Sheep 160 Pig 133 Environmental 289 from The Netherlands, UK and Switzerland 1983 – 2007	1,208 from The Netherlands 2000 - 2011	*C. jejuni & C. coli*	68 chicken 24 cattle 6 environmental 2 sheep and pig	Yes	Power analysis to determine minimum source isolates required and effects of using non-local and non-recent source data.		(411)
Asymmetric Island	Chicken 625 Cattle 168 Sheep 168 Pigs 160 Pets 133 Environmental 289 from The Netherlands, UK, Switzerland Apr 02 - Apr 03	737 from The Netherlands	*C. jejuni & C. coli*	77 chicken 18 cattle 2 environmental 2 sheep 1 pig		25% attributed to pet dogs and cats if included. Then 63% chicken, 11% cattle	Risk analysis: Dog, especially puppy owners, at increased risk of pet-associated infection	(412)
Asymmetric Island	Chicken 142 Turkey 23 Duck 11 Guinea fowl 2 Unspec poultry 26 Environmental 208 Ruminant 107 Pig 2 Other 5 from Luxembourg, The Netherlands, Belgium	1,153 from Luxembourg Dec 10 – May 13	*C. jejuni*	58.8 chicken 36.3 ruminants 4.9 environmental 0.2 swine		Combined case-control and source attribution study	Risk factors: contact with garden soil, consuming beef, poultry consumption in wintertime, water provider association with ruminant types	(310)
Asymmetric Island	Danish chicken 185 Imported chicken 137 Turkey 96 Duck 70	406 from Denmark 2007 - 2008	*C. jejuni*	52 Danish chicken 17 imported chicken 17 cattle		*flaA* Travel-related cases estimated at 2%.	*flaA* inclusion did not significantly increase discrimination	(146)

	Cattle 171 Pig 4 from Denmark and other EU countries 2007-2008							
Asymmetric Island	558 from Italy 6,854 from PubMLST (European) (detail not available)	31 from Italy 2012	*C. jejuni*	69.8 chicken 8.2 cattle 5.3 small ruminant 7.3 wild bird 6.2 environmental 2.9 pork		PFGE		(413)
Asymmetric Island	Chicken 210 Cattle 168 Sheep 160 Pig 133 Environmental 289 from The Netherlands, UK, Scotland and Switzerland 1990-2006	696 from The Netherlands 2002-2003	*C. jejuni*	66.1 chicken 21.2 cattle 10.2 environmental 2.4 sheep 0.01 pigs				(134)
Asymmetric Island	Chicken 275 Cattle 95 Sheep 136 Environmental 70 from NZ Mar 05 - Feb 08	502 from NZ Mar 05 - Feb 08	*C. jejuni*	76 chicken 18 cattle			As above	(414)
Asymmetric Island	Chicken 515 Cattle 282 Sheep 160 Pig 30 Wild bird 44 Rabbit 20 Bathing beach 71 Environmental water 23 from UK, Europe, Senegal, USA, NZ 1990 – 2000	1,231 from NW England Jan 00 - Dec 02	*C. jejuni*	56.5 chicken 35.0 cattle 4.3 sheep 2.3 wild animals 1.1 environment	Yes	Yes – of human population using linked and unlinked models which informed use of linked model of analysis		(139)

Asymmetric Island	467 from NZ Mar 05 - Feb 08	502 from NZ Mar 05 - Feb 08	*C. jejuni*	75 chicken 17 cattle 4 sheep 2 wild bird <1 water				(415)
Asymmetric Island	chicken102 turkey 6 cattle 17 sheep 32 environmental 11 from New Zealand 2009 – 2014	47 from New Zealand Mar 05 – Dec 14	*C. coli*	38 chicken 55 ruminants 7 environmental				(416)
Asymmetric Island	Chicken 111 Ruminant 2 Duck 1 Environmental 134 Pig 60 Turkey 27 Other 4 from Luxembourg, The Netherlands, Belgium	136 from Luxembourg Dec 10 – May 13	*C. coli*	82.4 chicken 8.8 ruminants 4.5 environmental 4.4 swine		Combined case-control and source attribution study	As above	(310)
Asymmetric Island	As above – not detailed by species	41 from The Netherlands 2002-2003	*C. coli*	70 chicken 12.2 cattle 8.9 environmental 5.0 sheep 4.9 pigs				(134)
Asymmetric Island	Chicken 514 Cattle 98 Sheep 54 Pig 380 Turkey 110 Riparian 67 from Scotland Jun 05 - Sept 06	500 from Scotland July 05 - Sept 06	*C. coli*	57 chicken 41 ruminants 1 swine 0.5 turkey 0.5 riparian	Yes			(153)
Asymmetric Island	Chicken 459 Cattle 86 Sheep 57 Pig 322	504 from Scotland July 05 - Sept 06	*C. coli*	56 chicken 40 sheep 2 cattle <1 pigs	Yes			(142)

	Turkey 111 from UK, USA, Europe, NZ 1990 – 2006			<1 turkey				
Population STRUCTURE	Chicken 715 Cattle 262 Sheep 293 Wild bird 188 Pig 40 from Scotland 2001 – 2012	172 from Scotland 2001	*C. jejuni & C. coli*	47.7 chicken 19.5 cattle 11.2 sheep 19.6 wild bird 1.8 pig		Case-case, case-control, time series analysis, genetic diversity and genetic distance.		(145)
Population STRUCTURE	Chicken 715 Cattle 262 Sheep 293 Wild bird 188 Pig 40 from Scotland 2001 – 2012	1,452 from Scotland 2005 - 2007	*C. jejuni & C. coli*	43.8 chicken 22.5 cattle 14.2 sheep 17.5 wild bird 0.03 pig		Case-case, case-control, time series analysis, genetic diversity and genetic distance.		(145)
Population STRUCTURE	Chicken 715 Cattle 262 Sheep 293 Wild bird 188 Pig 40 from Scotland 2001 – 2012	1,292 from Scotland 2010-2012	*C. jejuni & C. coli*	48.7 chicken 12.7 cattle 26.8 sheep 9.2 wild bird 2.6 pig		Case-case, case-control, time series analysis, genetic diversity and genetic distance.		(145)
Population STRUCTURE	Chicken 21 Cattle 22 Pig 23 Sheep 31 from Scotland Apr 06 – Mar 08	4,743 from Scotland July 05 - Sept 06	*C. jejuni & C. coli*	56* chicken 20 cattle* 18 pig 13 sheep	Yes		*% strains from liver of each source species identical to the 10 most predominant human genotypes	(417)
Population STRUCTURE	Chicken 277 Cattle 104 Sheep 97 Pigs 27 wild birds 175 from Scotland Jun 05 - Sept 06	202 from rural children Scotland 2000 - 2006	*C. jejuni & C. coli*	19 chicken 42 cattle 24 wild birds 12 sheep 3 pigs			Rural children <5 years	(147)

Population STRUCTURE	Chicken 277 Cattle 104 Sheep 97 Pigs 27 Wild birds 175 from Scotland Jun 05 - Sept 06	76 from urban children Scotland 2000 - 2006	*C. jejuni & C. coli*	43 chicken 35 cattle 6 wild birds 15 sheep 1 pig			Urban children <5 years	(147)
Population STRUCTURE	Chicken 435 Dog 134 Cattle 73 from Switzerland 2003 – 2014	351 from Switzerland 2009	*C. jejuni*	44 chicken 36 cattle 20 dogs		*flaB* attribution*:* 68% chicken, 18% cattle, 14% dogs		(418)
Population STRUCTURE	Poultry 742 Cattle 582 Sheep 217 from UK 2001 - 2006, Wild birds 921 from UK, Sweden, Australia 1999 – 2006	5,618 from UK 2003 - 2013	*C. jejuni*	2.1 – 3.5 to wild birds			Detail of wild bird attribution by family available	(406)
Population STRUCTURE	Chicken 257 Cattle 87 Water 266 Wild bird 63 from Canada Jul 05 – Oct 07	178 from Canada Jul 05 – Oct 07	*C. jejuni*	64.5 chicken 25.8 cattle 7.4 water 2.3 wild bird		Rural vs urban Case-case	Risk 1.89 times higher in rural areas. Two independent risk factors: occupational exposure to cattle, consumption of private well water	(419)
Population STRUCTURE	Chicken 435 Cattle 23 Dogs 159 from Switzerland 2002 – 2012	649 from Switzerland 2002 - 2012	*C. jejuni*	69.3 chicken 21.2 cattle 9.5 dogs	Yes			(144)
Population STRUCTURE	999 from Scotland Jun 05 - Sept 06 2,420 from PubMLST (details not available)	3,451	*C. jejuni*	46 chicken 31 ruminants 1.9 wild bird (20.7 unassigned <95% probability)	Yes	Case-case logistic regression.	Poultry associated cases more likely than ruminant to be adult, female, urban and in winter.	(143)

Population STRUCTURE	Chicken 1,288 Cattle 586 Wild bird 170 Environmental91 Sheep 249 from UK, USA, Europe, NZ 1990 – 2006	4,743 from Scotland July 05 - Sept 06	*C. jejuni*	58 chicken 38 ruminants 4 wild bird & environment	Yes; 70 chicken 84 ruminants 54 wild bird 38 environmental			(142)
Population STRUCTURE	Chicken 175 Pig 256 Cattle 24 from Switzerland 2003 – 2014	32 from Switzerland 2009	*C. coli*	76 chicken 16 cattle 8 pig		*flaB* attribution: 94% chicken 6% pigs		(418)
Population STRUCTURE	Chicken 459 Cattle 85 Sheep 57 Pigs 322 from Scotland Jun 05 - Sept 06	307 from Scotland Jun 05 - Sept 06	*C. coli*	40 chicken 41 sheep 14 cattle [3 pigs?]		Case-case	Female gender a risk factor	(420)
Population STRUCTURE	Chicken 175 Pigs 360 from Switzerland 2002 – 2012	81 from Switzerland 2002 - 2012	*C. coli*	87 chicken 13.6 pigs	Yes			(144)
Population STRUCTURE	Chicken 459 Cattle 86 Sheep 57 Pig 322 Turkey 111 from UK, USA, Europe, NZ 1990 – 2006	504 from Scotland July 05 - Sept 06	*C. coli*	40 chicken 40 sheep 14 cattle 6 pigs <1 turkey	Yes			(142)
Dutch Model	Chicken 275 Cattle 95 Sheep 136 Environmental 70 from NZ Mar 05 - Feb 08	502 from NZ Mar 05 - Feb 08	*C. jejuni*	58 chicken 19 cattle 12 environmental 11 sheep			The three models varied in precision but all attributed the majority of human cases to chicken.	(414)

Dutch Model	467 from NZ Mar 05 - Feb 08	502 from NZ Mar 05 - Feb 08	*C. jejuni*	52 chicken 17 cattle 10 sheep 5 wild bird 11 water				(415)
Modified Hald	Chicken 275 Cattle 95 Sheep 136 Environmental 70 from NZ Mar 05 - Feb 08	502 from NZ Mar 05 - Feb 08	*C. jejuni*	>58 chicken			he three models varied in precision but all attributed the majority of human cases to chicken.	(414)
Modified Hald	Danish chicken 185 Imported chicken 137 Turkey 96 Duck 70 Cattle 171 Pig 4 from Denmark and other EU countries 2007-2008	406 from Denmark 2007 - 2008	*C. jejuni*	38 Danish chicken 14 imported chicken 16 cattle		*flaA*	*flaA* inclusion did not significantly increase discrimination	(146)
Modified Hald	467 from NZ Mar 05 - Feb 08	502 from NZ Mar 05 - Feb 08	*C. jejuni*	67 chicken 23 cattle 8 sheep 1 wild bird <1 water				(415)

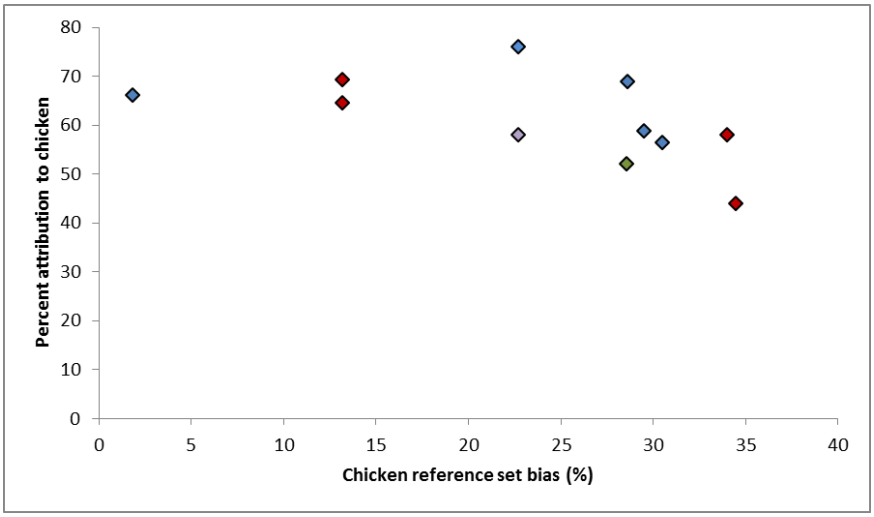


Figure 4.4. Proportion of isolates attributed to chicken in relation to the over-representation of chicken in attribution datasets (percentage higher than expected if all sources had been equally represented) by Asymmetric Island (blue), STRUCTURE (red), Dutch (lilac) and the Modified Hald (green) models.

Developing whole genome based attribution

4.18 Discriminatory power, the ability of a microbial characterisation method to identify two non-identical isolates as different, was considered in the second ACMSF report. This is one of the drivers for a move to WGS instead of seven locus MLST or other approaches. The limits of discriminatory power using WGS are close to the limits inherent in the rate of microbial genetic sequence change rather than being due to any feature of the method. These WGS data may allow detailed tracking of sources and spread (218) as well as offering the potential of more accurate overall estimates of source attribution. Whole genome sequence based schemes such as core genome MLST (cgMLST) (218) and single nucleotide variant schemes that can support analytical approaches to attribution using this fuller genetic data have been developed.

4.19 The use of sequence information across the whole genome is expected to increase accuracy in identifying sources since it has the potential to identify host associated subpopulations not identified by standard MLST across 7 genes (150). However, from initial work it appears that some *Campylobacter* strains can switch frequently between host species, surviving and replicating in different host species

without acquiring much if any host associated genetic sequence variation at each switch (239). Rather than using all available data analysed using a population genetic approach, some researchers are trying to identify informative genetic changes that can be understood from a biological perspective. Candidates include genes involved in vitamin B synthesis pathways (238) and antimicrobial resistance (144, 421). There are characteristics beyond genetic sequence that can be mined and may be informative, such as methylation (422), but the extent to which this will add useful information for source attribution is currently speculative. Attribution using WGS data is thus still in an early stage with some evidence for both promise and limitations. One limitation to the development and validation of methods using WGS for attribution is the lack of extensive sampled reference collections of isolates with WGS data. This is an area of rapid progress as regards numbers of sequenced isolates although not all are in well described coherent samples. The availability of schemes such as cgMLST is important to allow the curation and joint analysis of data from diverse sources to support WGS source attribution method development and validation.

Issues, opportunities and challenges

4.20 As genetic source attribution is increasingly applied a range of issues and opportunities for improvement have become evident. The relative lack of a complete or sophisticated sampling framework is evident in much source attribution work and is a source of potential bias and inaccuracy. Samples representing the chicken population have often been a mix of farm (i.e. reservoir), abattoir and retail isolates, for farm animals. Sampling of cattle and sheep is usually from their faeces on farm (i.e. reservoir) or raw milk as isolation rates from the carcases and retail meat, other than liver, are low for these animals.

4.21 Alongside analyses that consider the source of each isolate, well characterised types with strong host association may be used as sentinel types to estimate overall source attribution and may be another area for development not yet been explored for *Campylobacter*. Differences in virulence and survival in the food chain can create bias with both sentinel and unweighted approaches using all isolates. Although much human infection clearly comes from chicken there are some significant *C. jejuni* subpopulations on chicken (e.g. ST-661 complex) that cause disproportionately few

identified cases of human illness suggesting that these differences in survival or virulence may be a real rather than theoretical problem. Integrating information on differential survival or virulence into source attribution would decrease these biases. Similarly, immunity and cross-immunity could cause biases that if measured and adjusted for would improve accuracy. An additional issue in attribution is the large proportion of "generalist" subpopulations in human *Campylobacter* infections, such as ST-21 and ST45 complex isolates, which are present in a wide range of potential sources and may therefore be particularly prone to inaccuracy in attribution.

4.22 It is important to gain a better understanding of relevant behaviours of the different *Campylobacter* sub-types. ST45 is believed to be more resistant to environmental exposure than other STs and this needs to be confirmed. Similarly, it is important to determine whether the more robust *Campylobacter* types, that are most likely to reach the consumer on contaminated foods, show enhanced virulence.

Future directions

4.23 Work since the last report has demonstrated the usefulness of MLST-based source attribution. It has also highlighted the extensive work needed to ensure that these analyses are optimised, validated and supply intelligence to guide disease control. This experience highlights important future directions for improvements in genomic source attribution as WGS data becomes available covering: (i) isolate sampling; (ii) data handling; (iii) attribution method development and validation and, (iv) integration of these and other data to achieve provide useful information for policy and practice. In addition, applications to other pathogenic species in the genus *Campylobacter* may become increasingly important and feasible in the future.

Sampling

4.24 The widespread application of MLST coordinated through the PubMLST and later BIGSdb platforms allowed extensive reference datasets to be assembled opportunistically from these open access databases complementing those sampled prospectively in some attribution studies. Large well-sampled reference datasets of WGS data will be needed before the advantages of increased genomic data per isolate translate into reliable data and inference.

4.25 Although there is some work considering the impact of aspects of reference populations on accuracy, such as size and the closeness of temporal and spatial matching, this is not always used to guide sampling. How reference population sampling or selection considered these features was not usually reported in attribution studies and there was no evidence of an emerging methodological consensus around sampling. Future sampling and opportunistic reference population assembly should explicitly build on what has been learned already on these issues and emerging future evidence to optimise reference datasets.

4.26 Given the lag expected before WGS data and methods are ready for widespread use improved approaches to sampling for MLST studies remain current priority alongside sampling in WGS studies. Where structured longitudinal samples are important researchers, funders, and public health bodies need to maintain clear vision and sustained commitment to secure these.

Data handling

4.27 Large shared international databases such as BIGSdb and systems to share data across databases are essential to continued progress in this area. With the advent of WGS data these become necessarily more complex as regards genomic data handling. In addition, usefully indexed metadata, describing each sample, isolate, and collection add substantial utility but can be even more challenging to capture and manage than genetic data. The quality of these infrastructure developments and collaborative approaches across the field may have a stronger influence on the rate of development and application than technical developments in sequencing and analysis. Clarity on the minimum set of metadata in performing and validating source attribution analysis could support informed choices on what data to capture and store.

4.28 Schemes to organise and query genetic data are needed to make developing WGS resources accessible. Core genome MLST (cgMLST) [PMID: 28446571] gives one framework to extend approaches further, albeit within this relatively conserved set of genes. For gene-by-gene approaches (100), establishing the pan-genome of *Campylobacter* is important for a wide range of applications including source attribution. Using information on variation across the full range of genes could be expected to be far more accurate in identifying host specific lineages than analysis restricted to those common to most isolates. For SNP based approaches hierarchical

analyses are likely to be needed (423) requiring good quality genomes across the *Campylobacter* phylogeny to support this. As attribution methods develop, identification of which *loci* or SNPs are most informative for attribution may steer other aspects of data handling and scheme development to support quality assurance and access to these genetic data.

4.29 The range of data available for use, as well as analytical approaches to using it, is likely to mean an initial expansion in method diversity rather than standardisation. There may be an iterative role for integrating emerging consensus approaches and reference attribution datasets within the main databases to support standardisation and comparability as the field develops.

Attribution methods

4.30 The high utility of MLST-based attribution is accompanied by a striking lack of incremental optimisation in this area. Methodological standards should include reference dataset assembly based on the best available knowledge on the relative importance of sample size and matching of features such as time and place alongside feasibility and practical constraints. Reporting standards should include validation of attribution of known isolates using the study reference datasets and, where appropriate, sensitivity analyses of the impact of inaccuracy identified on the results. Approaches to correcting for identified biases are needed to support effective sensitivity analysis. Extensive direct comparison of algorithms and their application is another striking gap in the published literature. These approaches to optimising MLST-based attribution are important to support policy and practice now and can provide a template for optimising methods based on more extensive data.

4.31 The twin challenges of identifying optimal attribution methods and the best data to use are substantial and inter-related. The choice of genes for MLST schemes sought relative homogeneity and neutral selection by using housekeeping loci, an option that is unlikely to be optimal in the future where using a large proportion of WGS data will require analysis across very different genes following different evolutionary trajectories, and likely including the accessory genome. One approach is to build up understanding of those *loci* which provide information on host source, and how best to combine loci under different forms of selection. The examples above of vitamin B5 biosynthesis and antimicrobial resistance illustrate this approach. More empirical

mathematical approaches using all available genetic data are also possible, although these will meet issues of computational power requirements as well as any independence assumptions between genetic markers becoming increasingly unrealistic when using WGS data (424). The STRUCTURE and asymmetric island models would not be practicable to run on large whole genome datasets. Additionally, there may be interactions between some loci further complicating how information should be combined across the genome. The fastStructure variant of STRUCTURE (425) may be more efficient but overall this is an area where the application and adaptation of developing population genetic analysis algorithms is likely to need to be updated for some time.

Integration and application

4.32 Few studies have sought to combine genetic and other data in source attribution. Integration of genetic attribution in models that take account of other data such as bacterial load in the food chain and virulence may improve accuracy and use for policy. This raises a range of methodological questions and also a need for empirical work to test effect sizes of factors, such as differential survival in the food chain. There are some data suggesting that the *Campylobacter* populations in cattle and sheep faeces are similar to those found in liver (417), but scope for a substantial increase in understanding in these potentially important areas.

4.33 A potential approach is to combine attribution and Quantitative Microbiological Risk Assessment (QMRA) as already considered for *Salmonella* (426). Currently QMRA treats all bacteria the same although in reality, each genotype will, to some extent, have different survival characteristics and virulence. These properties can be utilised to determine likelihood of survival from source, through the food chain to consumption and likelihood of illness. Another area for of integration is joint analysis with risk factor data. Source attribution has been used in conjunction with case-control data (134, 310).

4.34 Approaches to integration developed using MLST with non-genomic data, will support similar work with WGS based attribution as it develops.

Other species

4.35 Isolation procedures are generally optimised for *C. jejuni* and *C. coli* (427). Other members of the genus may therefore contribute a greater proportion of illness than is recognised, at least in some parts of the world (428, 429), with most evidence to date for *Campylobacter upsaliensis* (430, 431) for which the clinical picture may also be more severe with for example bacteraemia occurring more commonly (430). More recent reports indicate that *Campylobacter concisus* may be a significant cause of human bacterial gastroenteritis (429). The advent of genome based molecular tools may support improved detection of a wider range of other species in human samples and in foods (432) and clarify which of these are associated with disease in humans. Partial genome sequencing approaches initially applied to *C. jejuni* (121) and *C. coli* (109) were later extended across other species (122). Similarly, although this chapter focuses mainly on *C. jejuni* and *C. coli* the general approaches and issues for the future described are also applicable across other species. Similar approaches are likely to contribute to the identification of other species in potential sources (432) as well as in cases of human infection.

Conclusions on what is known

4.36 Genetic sequencing technologies have removed issues such as reproducibility and non-typeable strains and all *Campylobacter* isolates can now be reliably characterised in a way that is mainly only limited by the actual level of existing biological variation. This new form of isolate characterisation goes beyond assigning a type and allows more sophisticated analysis of relationships between isolates, including quantitative prediction of whether isolates are from a particular source. Analysis that takes into account the biology sustaining populations of bacteria and transmission between these populations allows more accurate information to guide risk assessment and management. The benefits available from shared large and structured reference datasets has become increasingly clear; however it has also become clear that there is substantial transmission among some of the animal populations that act as reservoirs for human *Campylobacter* infection, particularly between farm animal species, and that this limits accuracy of identifying source of human infections from these groups of *Campylobacter* that are shared across several species using the best large scale datasets currently available.

Remaining uncertainties

4.37 We do not know whether the more detailed data offered by WGS, if applied at scale and correctly analysed, can allow accurate attribution of isolates that appear to be shared across several host species when observed using the detail available from 7-locus MLST. Our ability to analyse large scale datasets of WGS data are both methodologically and computationally limited. Alongside these more speculative research questions, more clearly discernible issues include the lack of standardised approaches to reporting genetic source attribution that integrate validation of the approach used and sensitivity analysis. There is also a lack of synthesis of the evidence available from current data on virulence including the differential survival of isolates in the food chain or other routes to human infection. An extension of this is the wider and more difficult challenge of integrating large scale genetic data and analysis with other information to allow more accurate mapping of transmission.

Recommendations

Risk assessment

4.38 The valuable contribution made by genetic source attribution to estimating the relative contributions of different sources to human disease could be enhanced by (i) validating and optimising accuracy from existing methods and data; (ii) applying reporting standards explicitly report validation results and present adjustments or sensitivity analyses, to include the impact of inaccuracies or uncertainties identified by validation.

4.39 Establishing well sampled and validated datasets, with the appropriate metadata within the larger less structured genetic data databases are essential to provide reference data for source attribution. Planning and initiating collection of these data is needed to ensure that this resource will be in place when needed and to maintain longitudinal data to support analyses that consider change over time and provide intelligence to guide risk management.

Research

4.40 Developing and testing source attribution methods that utilise informative data across the whole genome as the cost of these data falls and availability increases is needed to optimise and identify the limits to these data and this approach.

4.41 Genomic attribution should be integrated with other approaches to maximise its value. This includes: (i) combining source attribution analysis with epidemiology and risk assessment; (ii) use in integrated *Campylobacter* surveillance across animals, food and humans; and (iii) sampling studies to support this work.

Chapter 5: Risks in the Food Chain - Poultry

Introduction

5.1 Several different foods have been associated with outbreaks of campylobacteriosis. To prioritise the risk presented by such food groups, a simple risk rating has been developed using the criteria defined in Table 5.1. Each of these food groups will be considered in more detail in this Chapter and in Chapter 6.

Table 5.1: Relative qualitative risk of different foods

Food	Prevalence	Levels	Outbreaks	Volume consumption	Risk rating (multiplied)
Raw poultry	5	5	4	5	500
Raw meat	2	3	2	5	60
Raw milk	3	3	4	1	36
Prepared salad vegetables	1	2	2	4	16
Water (untreated ground / surface)	2	2	2	2	16
Raw milk cheese	1	2	2	2	8

1= Rarely present (<1%); Always low levels (<10per g / ml) if present; Rarely associated with outbreaks; Very low volume consumption
2= Occasionally present (1-10%); Mostly low levels; Occasional association with outbreak; Moderate volume consumption
3 = Often present (11-50%); Occasionally high levels; Associated with outbreaks; Average volume consumption
4= Usually present (>50% 70%); Usually high levels; Often associated with outbreaks; High volume consumption
5 = Always present (>70%); Always high levels (>100per g / ml); Always associated with outbreaks; Very high volume consumption

***Campylobacter* in broiler chickens: commensal or pathogen?**

5.2 *Campylobacter* spp. were identified as a cause of enteritis in humans in the 1970s and it soon became apparent that contaminated chicken meat was an important source and/or vehicle for infections. It is now implicated, either directly or indirectly, in up to 80% of human *Campylobacter* infections (433) but the percentage ascribed to this food source varies according to analysis methods used.

5.3 Most consumers will eat chicken meat from birds grown in intensive systems (broilers) and these animals account for 31% of global non-indigenous meat production. Over 6 billion (bn) broilers are grown in the EU each year, with ~1bn being grown in the UK, and this industry is vital to the rural economy and as a source of low cost, nutritionally high-quality protein; the value of EU-28 poultry meat output exceeds €21bn per annum.

5.4 There is a growing global demand for broiler chicken meat, which can only be fully satisfied by using broiler systems that may affect bird health, welfare and performance, as discussed below. It is essential that disease and poor welfare, which can compromise productivity, are better controlled and associated antimicrobial use is reduced. It is recognised that the UK poultry industry has made improvements in recent years in reducing the use of antibiotics.

Commented [MU1]: Text has been added to reflect Moy Park consultation response.

In this chapter we present data, from 1981 onwards, that indicate that not only do *Campylobacter* more easily infect birds with poor health and welfare they can also be a cause of it and can compromise production efficiency. Data show that *Campylobacter* spp. are not always the harmless commensals in broiler chickens that they are still purported to be (434), even in light of much contrary evidence.

5.5 It is important to remember that when published data on the interactions of *Campylobacter* and chickens are analysed the observed outcomes of infection can be governed by the:

- *Campylobacter* strain used, the infective dose and route of infection;
- bird type infected and its age
- environment the host and the pathogen share.

In simple terms, the above means that with some strains of *Campylobacter* there are no obvious external signs of disease while with others the bird may suffer intestinal damage and diarrhoea.

5.6 It cannot be stated too strongly that the behaviour of one *Campylobacter* strain may not be representative of the whole bacterial population. Bird type can also have an impact on infection dynamics.

5.7 Contaminated chicken poses two health threats: surface contamination and infection of edible tissues. The many outbreaks in the UK caused by contaminated liver, and dishes derived from it, is testament to the latter. *Campylobacter* infection also compromises chicken health, welfare and performance. Only the public health risk from carcass surfaces can be addressed during processing. The industry should be encouraged to continue their efforts to control *Campylobacter* on-farm and supported by targeted research, as appropriate. The summer peak of chicken infection is a particular area of concern and needs to be addressed. It is unlikely that a cost-effective, easy to deliver vaccine will be available in the short to medium term and the development of 'resistant' chickens remains a long-term goal.

The resistance of *Campylobacter* to the extra-intestinal environment:

5.8 There is a long-held view (435), that *Campylobacter* are highly sensitive to stresses such as heat. There is no doubt that in broth challenge models *Campylobacter* spp. are more heat sensitive than other foodborne pathogens such as *Salmonella* spp. and *Listeria monocytogenes*. Data of this type have been used to determine risks and the impact of interventions. It is important to recognise that death rates in broth do not necessarily reflect those in foodstuffs and laboratory experiments may not take into account the impact of environmental interactions. Work in the past 10 years has shown that, unlike *Salmonella* and *E. coli*, exposure to low temperature does not increase the heat sensitivity of *C. jejuni*. It is also now clear that cells of *Campylobacter* attached to chicken tissues can show much higher levels of heat resistance than previously thought (35). C*ampylobacter* is also capable of long-term survival at chill temperatures and in the environment provided the conditions are moist. In contrast, *Campylobacter* is highly sensitive to desiccation.

Production systems

5.9 In broiler systems birds are housed for their entire life usually at a final stocking density of 38Kg/M^2. Modern, rapidly growing broiler breeds can reach slaughter weight (2.2Kg) at around 36 days of age, although slower-growing breeds reared in 'high welfare' systems and stocked at a lower final density, usually 30Kg/M^2, reach slaughter weight at around 50 days of age. Chickens are also produced in the UK using extensive free range and organic systems, where the birds spend approximately 50% or most of their life with access to the outside, depending on the system used. Extensively reared birds comprise only a small fraction of the total retail market.

5.10 The modern broiler chicken is a very different animal from that of ~60 years ago and has been bred selectively to increase weight gain, muscle mass and food conversion efficiency. A seminal paper on the changes in chicken as a result of selective breeding is that by Zuidhof et al (436). In this paper, the authors compared birds from 1957, 1978 and 2005. Using average live weight as an indicator of change it was shown that at 28 days of age: the 1957 birds weighed 316g, the 1978 animals were 632g and the 2005 birds had a weight of 1396g. The 2005 birds were 440% heavier than their 1957 counterparts at this time point.

5.11 It is important to better understand the animal and public health consequences of such changes. Chicken production should be sustainable, with a safe product and good bird welfare. The international poultry industry can find this difficult to achieve on occasions and birds can experience poor welfare, often in the form of pododermatitis and hock burn marks, contact dermatitis of the feet and lower legs respectively. These problems relate mainly to poor gut health, manifesting as diarrhoea, causing wet litter, which ferments, becomes corrosive, resulting in the skin lesions. A variety of factors have been shown to affect the incidence of contact dermatitis, including the house environment (437-439), use of antibiotics and season (440) and bird breed (441, 442). FSA- and BBSRC-funded work in the UK in 2003-6 (443, 444) found that there can be marked flock-to-flock differences in observed incidences of health markers in commercial production systems.

Commented [MU2]: Four words added to reflect Moy Park's consultation comments.

In vivo behaviour of *Campylobacter* in chickens and the public health threat

5.12 Despite much research, carriage rate in chickens and numbers of human cases remain high in the UK and many other countries across the globe. There is long-standing and mounting evidence that the health threat from *Campylobacter*-positive chickens may not be only from surface contamination; edible tissues are also positive, almost exclusively with *C jejuni*, (445); (446, 447) (448, 449) and contaminated chicken liver is a major vehicle for human infection (284), as is undercooked flesh. Contamination of chicken liver and muscle with *C. jejuni* is a public health threat that seems to be increasing but is poorly understood. Levels in livers can be high (445) and this can be associated with disease (448). This section describes work to examine the factors influencing the *in vivo* behaviour of *C. jejuni*, in particular, and the human and chicken health threats these behaviours pose.

5.13 The importance of chicken in human *Campylobacter* infection has resulted in many studies being undertaken over the last 30 years. Most work on chickens and *Campylobacter* has been from a public health perspective, largely with a focus on lowering levels in the gut in order to reduce numbers on carcass surfaces. As a consequence, few studies on artificial infection have looked outside colonisation of the caeca.

5.14 Much of the work on interventions to lower the perceived public health risk of surface-contaminated carcasses has been driven by a paper published in 2003, (435), which estimated that a two log reduction in levels of *Campylobacter* on chickens would have a huge public health benefit. There are potential problems with this approach. The above paper (435) underestimated the heat resistance of *Campylobacter* because relevant data to properly assess this were not available at that time. The perceived high sensitivity of *Campylobacter* spp. to heat meant that Rosenquist *et al* (435) studies did not reflect cooking of chicken and or chicken pieces and essentially dismissed tissue contamination and under-cooking as public health threats. This model has essentially remained unchallenged and given the international importance of infected chicken tissues, discussed above, it needs to be reassessed, as do the control strategies derived from it. Recent work has shown that

Campylobacter is more heat resistant on chicken than thought previously and this is discussed in detail in section 5.8.

Routes of contamination of chicken tissues

5.15 The routes of contamination of chicken liver and muscle are yet to be fully explored and/or explained. The former may well be disease-associated, as *Campylobacter* can cause the disease Vibrionic Hepatitis in chickens and past work has found an association with the appearance of disease in liver and high numbers of *Campylobacter* in the tissues of this organ (448). Contamination of muscle tissues may be associated with septicaemia or bacteraemia in chickens. Figure 5.1a below from a paper published in 1984 (450) shows that strains of *C. jejuni* can readily spread from the chicken gut and in the study quoted, ~15% of the artificially infected birds had *Campylobacter* in their blood. More recent work in the USA found that 12% of blood samples taken aseptically from naturally infected broilers at slaughter were *Campylobacter*-positive (451). It is possible that when the animals are bled at slaughter *Campylobacter* lodges in the small blood vessels in muscle tissues. More attention needs to be paid to preventing the contamination/infection of edible tissues, which cannot be controlled in the slaughterhouse.

5.16 One of the problems common to most types of research is that when published work is quoted people focus on the results and pay rather less attention to the techniques used. This is particularly important when examining the infection dynamics of *Campylobacter* and chicken. In published work examined for this report, studies used a variety of *Campylobacter* strains and different types of chicken,

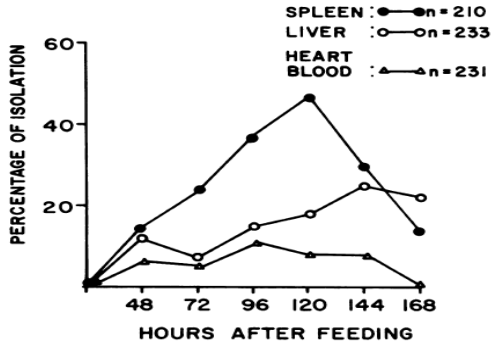


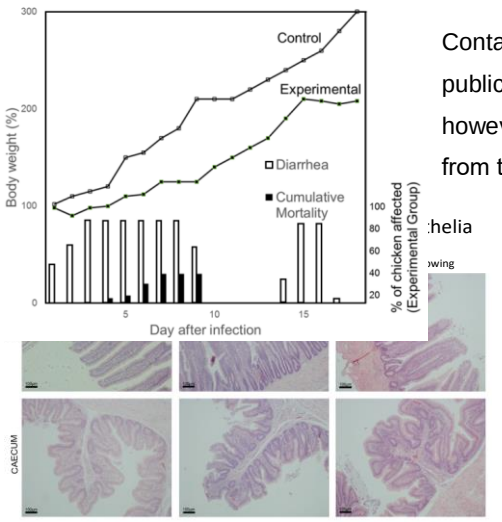
Fig 5.1a Systemic invasion after feeding of live *C. jejuni* organisms in chicken

including both broilers and layers. As we will show later in this chapter, the outcomes of infection and the *in vivo* behaviour of the bacteria, can be strongly influenced by bacterial strain, bird type and the environment they share. Thus, one *Campylobacter* strain may not be representative of the population as a whole and commonly used commercial broiler types show quite different innate immune responses to infection (452). This is discussed in more detail below.

***Evidence for Campylobacter* as a pathogen in chickens**:

5.17 *Campylobacter* is the major, principally chicken-associated, zoonotic pathogen in the EU, infecting an estimated 9 million people annually at an approximate cost to the EU economy through impact on public health systems and lost productivity of €2.4 billion (453). Contaminated chicken has a much greater public health impact than red meat; however, this may have distracted attention from the bacteria's evolving role as a chicken pathogen. There is strong evidence from laboratory studies over 30 years (450, 452, 454, 455)showing that *Campylobacter* can have a negative impact on broiler gut health (Fig. 5.1b) and thus on welfare and performance (Fig. 5.1c); *for the sake of clarity, this figure, which is from a paper published in 1981 by Ruizpalacios et al 1981, was re-drawn using the original data*).

Fig 5.1b and Fig 5.1c: *C. jejuni* affects broiler weight gain and health



In this study, approximately 90% of the birds had diarrhoea, there was cumulative mortality of around 40% and the final body weight of the infected birds was around 30% lower than the controls. Recent UK work (452) supports the earlier studies on gut damage and also shows that *Campylobacter* strains differ in impact on chickens (456) and this creates difficulties when studies are compared, especially given that broiler chicken strains show differences in innate immune responses to *C jejuni*

(452). Recent work (457) has also shown that the innate immune responses of laying hens to *Campylobacter* differ from those of broilers.

5.18 Given the work quoted above, which is consistent with publications by many research groups that chickens infected artificially with *Campylobacter* can suffer poor health and welfare, these bacteria would perhaps be better classed as opportunistic pathogens and not chicken commensals.

5.19 It is important, particularly for engagement with the poultry industry, that work using artificial infections is supported by studies in commercial systems. Field work in the UK, including that funded by FSA (443, 444) found significant relationships between broiler health/welfare and *Campylobacter* infection of commercial flocks. Neill *et al*. (458) in Northern Ireland found that infection of commercial flocks at any age was associated with wet litter, and in birds infected at <2 weeks old, there was raised mortality. The later UK work (443, 444) produced similar data with ~800 pre-thinned commercial flocks from 214 farms and three poultry companies. The companies made all health and production data for each flock available to the research team. Data were analysed using random effects logistic regression and/or generalised linear mixed models. The outcomes of this work were essentially in agreement with the earlier observations of Neill et al (458) that flocks with high levels of contact dermatitis and/or condemnation for infection at slaughter (mainly avian pathogenic *E. coli*, APEC) were more likely to have *Campylobacter*. US work around the same time also showed a link with APEC (459).

5.20 Subsequent Defra-funded UK work compared effects of *Campylobacter* infection in 'fast' (2.2kg in ~35 days) and 'slow' broilers (2.2kg in ~50 days) (460). Birds were given the same diet and reared at the same density. Post-infection, 'fast' birds had wet litter and high levels of pododermatitis. 'Slow' birds had dry litter and little pododermatitis. It was not recorded if birds had diarrhoea. This was the first study to show that *Campylobacter* affect broiler types differently (460).

5.21 Subsequent work (452) using the same *C. jejuni* strain and bird types as the above study, found that 'fast-growing' chickens had severe diarrhoea whereas 'slow-growing' birds did not. There was much damage to gut mucosa with 'fast' birds,

particularly in the ileum (Fig. 5.1c). In ‘slow’ birds, with normal faeces, the villi were less affected (Fig. 5.1c). The bacterial mechanisms for such effects were not determined and such work needs to be undertaken. Preliminary analyses of innate immune responses (452) showed that gut damage in ‘fast’ birds was associated with dysregulation of pro-inflammatory cytokines. Work by another group, using ‘fast’ birds and a different *C. jejuni* strain, found similar damage to ileal mucosa but did not report diarrhoea (455). Damage to gut mucosa has also been reported in some other studies (450, 454, 461), but not all (462) (463). Awad et al (464) found that infection compromised gut barrier function and performance and host nutrient uptake Awad et al (455), which is consistent with the damage to the ileum shown in Figure 5.2. Other studies report a range of impacts of infection with *C. jejuni*. As with Awad et al (455) and Humphrey et al. (452), other work has reported differences in whether birds suffered diarrhoea or not. Sanyal et al.(450), Ruizpalacios et al. (454) and Sang (465) reported this, particularly in young chickens, but others did not. Other work found that infection of young birds (<3 days of age) with *C. jejuni* was lethal (466) and this has also been seen in turkey and ostrich chicks (467, 468). The data indicate that bird type impacts on the effects of *Campylobacter* but bacterial strain is also important. These, and earlier data, show that *Campylobacter* can be chicken pathogens although strains behave differently. A working hypothesis could be that: “*Campylobacter* in chickens contain genotypes that are pathogenic (harmful) and others that are less so (i.e. benign to chickens)”. It is possible that the ‘harmful’ strains are more likely to be ‘invasive’, leaving the chicken gut and infecting edible tissues in birds. *Campylobacter* colonisation starts with low-level invasion of gut mucosa (469). With some strains this may be more aggressive. There are now powerful economic and bird welfare reasons for better *Campylobacter* control on farm.

***Campylobacter* and commercial broiler performance**

5.22 Many factors affect broiler performance, including breeder flock age and stocking density (470), season (471), and light intensity (472). Good gut health in broilers is key to better growth rate and feed efficiency (473). Given how *Campylobacter* can affect the gut (Fig. 5.1b and 5.1c) it is no surprise that infected birds may have poorer performance. An Indian study (474) compared broiler flocks with good and poor feed conversion ratios (FCRs). Using metagenomics to

characterise gut microbiota, it was found that in birds with good performance (low FCR) the % of sequences assigned to *Campylobacter* was 0.05, whereas in poorly performing flocks (high FCR) it was 12. This means a highly significant 240-fold difference in the presence/levels of *Campylobacter* between the flock types. In 2014, with UK 'fast-growing' commercial flocks (>150 in total), the impact of *Campylobacter* on FCRs was examined. The average economic impact due only to *Campylobacter*-associated poorer FCR was high at ~£25 per 1,000 birds in infected flocks (Sparks et al, personal communication), even when all confounding factors had been removed and the differences between *Campylobacter*-positive and -negative flocks was highly significant ($p<0.01$). Recent work in Ireland found that high-performance birds (low FCRs) had both a lower *Campylobacter* prevalence and a higher gross income per bird. An important risk factor for high FCR was poor biosecurity and its impact, which could be *Campylobacter*-related, was ~€100 per thousand birds (475). Given the size of the UK industry, potential losses due to *Campylobacter*-related poorer FCRs alone could be £millions per year. There are also other costs like wastage from *Campylobacter*-related mortality and culling. Preliminary analysis of published experimental data on the financial impact of reported reduced bird performance, but not mortality, due to *Campylobacter* was, on average, ~£25 per 1,000 birds, although in Fig. 5.3, from a 1981 paper, it was greater (454)

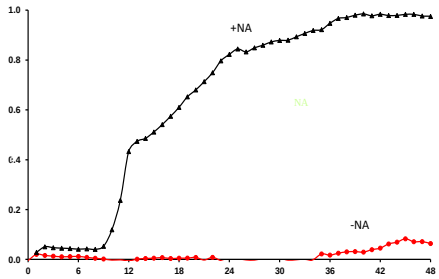
5.23 *Campylobacter* in commercial chickens comprise a diverse population and data show that some strains may pose a real risk to bird health and performance. Identifying the 'more chicken-pathogenic' strains and mechanisms by which they affect the birds is an important prerequisite for effective control and improving broiler welfare. Little is currently known about this, but clearly damage to gut mucosa and diarrhoea will affect nutrition and the former will also contribute to the spread of *Campylobacter* to edible tissues. Recent work found that nutrient transporter expression in the broiler gut was compromised by *C. jejuni* (455). This may have a negative effect on performance and down-regulation of mRNA expression of glucose and amino acid transporters may result in nutrient accumulation in the intestinal lumen, favouring *C. jejuni* replication (455). Increased gut damage and physiological disruption have clear implications for bird performance in a system with inherent low economic returns.

5.24 Broiler chickens can carry both *C. jejuni* and *C. coli* but it seems that the former poses the greater public and bird health risk. In an investigation in New Zealand (445), 168 out of 169 *Campylobacter* isolates from chicken liver tissues were *C. coli*.

Poor bird welfare and the *in vivo* behaviour of *Campylobacter jejuni*

5.25 Broiler chickens may suffer both chronic and acute stress. The former will result from poor in-house environments, leading to conditions like hock marks and pododermatitis as discussed earlier in this chapter. They may also be co-infected with poultry-pathogenic bacteria and viruses, which can change the *in vivo* behaviour of *C. jejuni*.

Fig. 5.1d: Noradrenaline increases C. jejuni growth in low iron media



5.26 The most important event in the life of a broiler chicken with regard to acute stress is likely to be depopulation. The birds will be: (i) denied food to allow a decrease in gut contents; (ii) picked up manually often with 4-5 birds in each hand; (iii) placed in crates and transported to the processing plant. Work has shown that numbers of *Campylobacter* are higher in broilers subject to de-population processes than in ones not enduring these procedures (445, 476, 477). This may be associated with the release of noradrenaline (NA) into the gut lumen in those animals subjected to acute stress. NA acts essentially as a siderophore for *Campylobacter,* allowing it to better compete for iron in the gut. Growth rates of *Campylobacter* cells exposed to physiological levels of NA are much more rapid than those of bacteria not exposed to the hormone (478); (Cogan *et al.*, 2007); Fig. 5.1d). De-population processes also change gut colonisation patterns of *Campylobacter,* with the bacteria being found the whole length of the intestinal tract rather than mainly in the caecum and colon (479) (480). *Campylobacter* in the upper gut (the ileum) seem better able to leave the gut and infect edible tissues (456)

5.27 Thinning, the removal of a proportion of the birds around 4-7 days before the rest of the flock is taken for slaughter, is common industry practice in the UK. In most studies, this event has been shown to approximately double the incidence of *Campylobacter*-positive flocks. Breakdown in biosecurity may well play the major role in this, but the stress the remaining birds experience as a result of the depopulation process may also increase their susceptibility to *Campylobacter*, as it does with *Salmonella* and facilitate extra-intestinal spread (481, 482).

5.28 Poor welfare may also increase inflammatory responses and/or cause immune dysregulation in the gut, affecting epithelia and resistance to *C. jejuni* translocation. In the presence of the cytokine interferon gamma, *C. jejuni* rapidly reduces tight junction integrity Rees *et al*. (483) Given the role of heterophils in limiting enteric pathogens to the chicken gut any reduction in function as a consequence of harvest stressors may facilitate *C. jejuni* extra-gut spread (484). Recent work where birds were given corticosterone, an immunosuppressant, and then infected with *C*. *jejuni* found that there was marked extra-intestinal spread of the bacteria (485). This may be a consequence of suppression of T cell replication.

The public health Importance of invasive *Campylobacter*

5.29 From a human health perspective, *Campylobacter*-contaminated chicken seems to pose two threats: high surface levels, a cross-contamination risk, and infection of edible tissues. It is important to recognise that the presence of *Campylobacter* on carcass surfaces may not be the only major health threat. A high percentage of livers from commercial chickens are now *Campylobacter*-positive (445) and this may be associated with genetic selection programmes to achieve faster-growing broilers. Chicken liver is a major vehicle in outbreaks, as discussed above; however, data show that undercooked chicken meat is the most important vehicle internationally for human infection and *Campylobacter* can be isolated from muscle (see earlier) (447). The damage to gut mucosa (Fig 5.1b facilitates extra-intestinal *Campylobacter* spread. Such spread to tissues is clearly a threat to human health when they are under-cooked, as many international investigations have shown (224, 486). It cannot be controlled in the poultry slaughterhouse and encouragement and support should be given to the UK poultry industry to continue to

maintain high levels of on-farm biosecurity and to continue to explore other on-farm interventions such as dietary additives to reduce gut inflammation.

Recommendations

5.30 We recommend that there is a need to better understand:

- **The population diversity of *Campylobacter* spp., principally *C. jejuni*, in terms of infection biology in chickens and impact on gut and general health, welfare and performance.**
- **The genetic mechanisms used by *C. jejuni*, in particular, to damage gut mucosa and spread from the intestine to edible tissues.**
- **Innate immune responses of different chicken types to different *C. jejuni* strains to inform vaccine development**
- **Innate immune responses of different chicken types to inform the selective breeding of more *Campylobacter*-resistant chickens.**
- **The role of gut microbiota in either preventing or facilitating the colonisation of that organ by *Campylobacter* spp.**
- **Wherever possible and practical, chicken infection studies should be done at a scale that is relevant to industry practice.**

Market statistics

5.31 Poultry is defined in EU Regulation 853/2004 (487) as farmed birds, including birds that are not considered as domestic but which are farmed as domestic animals, with the exception of ratites. This is generally considered to include chicken, duck and turkey. Poultry meat is the most commonly consumed meat in the UK representing approximately 42% of consumption (488). The consumption of poultry meat and chicken in particular has steadily increased over many decades principally due to it being a readily available and affordable source of protein. The UK per capita consumption of poultry meat has increased to 35.4kg/person/year, the highest level in over a decade (Figure 5.2).

Kg/person/year	Poultry meat	Pig meat	Beef & Veal	Total meat
2000	30.5	23.6	18.5	79.2
2005	31.8	24.7	20.7	83.4
2010	31.8	24.1	18.6	79.4
2015	35.4	25.0	18.1	83.7
Supplies available for consumption. All data in carcase weight equivalent. Source: AHDB Market intelligence calculations based on data from Defra, HMRC, ONS				

Figure 5.2. Poultry consumption in the UK 2000-2015

5.32 The UK is approximately 73% self-sufficient in poultry (488) and production is dominated by 4 major companies who account for over 80% of the supply. Production of poultry meat has increased significantly in recent decades and there were 953.1 million broiler birds slaughtered in the UK in 2015 (Figure 5.3)

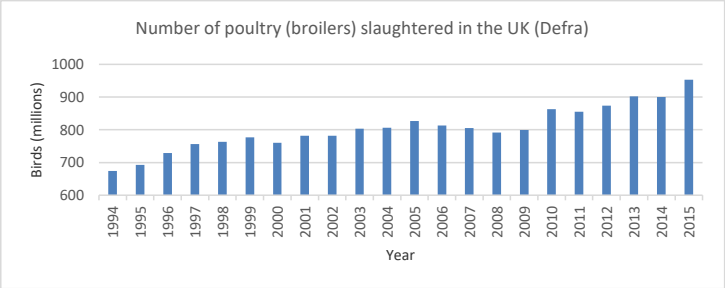


Figure 5.3 Poultry slaughter in the UK 1994-2015

5.33 The great majority of poultry slaughtered is for chicken meat and this represents over 80% of poultry meat produced in the UK (Figure 5.4).

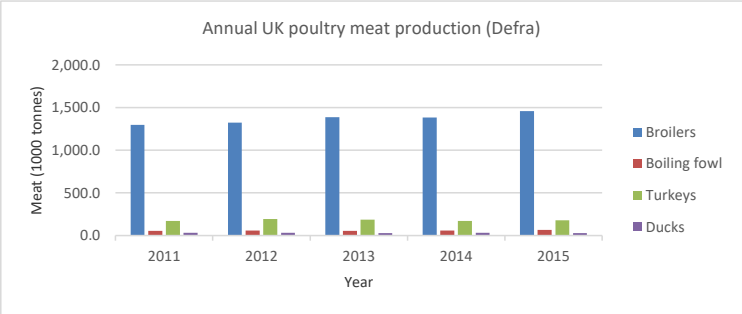


Figure 5.4. UK production of poultry meat 2011-2015

5.34 Chickens are reared predominantly for meat (broilers) and egg (layers) production although spent laying hens are also used for meat production at the end of their laying lives. Chicken meat is sold as chilled or frozen whole birds, portions e.g. thighs, wings, breast or as diced and minced (Figure 5.5). Portions may be sold with skin on or off. Chicken meat is also further processed i.e. flavoured, reformed, cooked and is used as an ingredient in many products.

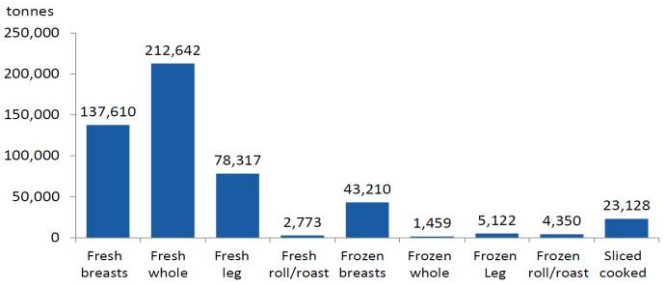


Figure 5.5. Volume sales of chicken products in Great Britain (2015) Source Kantar WorldPanel, from AHDB Poultry Pocket Book, 2016.

5.35 Although it is recognised that all poultry meat may be a source of *Campylobacter* spp. contamination and surveys have demonstrated a high prevalence in ducks, turkeys and chickens (489), the biggest impact on reducing human disease will be achieved by focussing on reducing contamination in chicken meat due to the volume of product consumed. This chapter will therefore focus on the production of chicken and associated controls.

Overview of chicken production

5.36 Broiler chickens are reared using two broad agricultural approaches: intensive systems (permanently in sheds) or extensive systems (in houses with access to external environments e.g. free range or organic). The breeds used for these systems together with the length of time of rearing differ. In all cases, the chicks used for broiler production are produced from parent flocks of cockerels and hens that produce fertilised eggs (Table 5.2).

5.37 The eggs are collected and transported to hatcheries where they are incubated until hatching. Day old chicks are transported to broiler chicken farms and placed in houses. These vary in construction depending on age and may have earth or concrete floors with wood, metal or concrete/brick walls. Multiple houses are present on most farms and prior to chicken placement they are usually cleared of chicken manure from the previous crop, cleaned and disinfected (including equipment in the house e.g. feeders, drinkers, etc.) and then made ready for the next flock with a suitable floor covering such as straw or wood shavings.

5.38 Intensive and extensively reared birds have access to houses, the difference being that the latter also have access to external environments in an enclosed farm. Birds are reared for time periods that vary depending on the desired size of the final bird and the production method and breed used. In general, intensively reared birds are grown for 5-8 weeks to achieve carcase weights of approximately 2kg whereas extensively reared birds would take several more weeks to achieve similar weights. Birds are provided with feed and water *ad lib* during rearing and litter remains in the house until it is emptied. Houses are ventilated from roof and window vents and have two entrances; large doors at one end to allow population/depopulation and small access doors to allow farmer/visitor entry. Houses have a perimeter of concrete/stone/earth to deter pests. Houses are not hermetically sealed units and can be accessed by external vectors of contamination including flies, other insects and rodents. Extensively reared birds will also be subject to vectors such as other birds and wild animals. Animal health and welfare is monitored throughout rearing including measurement of mortality, injury, disease and weight gain.

Table 5.2. Process flow diagram and technical considerations for typical raw chicken production (adapted from Bell and Kyriakides, 2009).

Process Stage	Notes
Agricultural / farm stages	
Great grandparent / Grandparent stock ↓	Birds bred to develop desired traits in breeders / broilers
Broiler parent breeder farms ↓	Cockerels and hens produce fertilised eggs
Broiler hatchery ↓	Eggs incubated to produce hatched day-old chicks
Broiler farm ↓	Day old chicks populated in shed(s) on farm
Broiler rearing ↓	Chickens reared for 5+ weeks depending on required weight and production system
Depopulation ↓	Birds removed from a shed in full or at varying proportions on more than one occasion (partial depopulation / 'thinning')
Transportation ↓	Birds transported in crates and modules to the abattoir
Abattoir ↓	Birds electrically or controlled atmosphere stunned prior to killing by severing carotid artery
Processing Stages	
Scalding & defeathering ↓	Feathers wetted in hot water (scald) tank prior to automated defeathering using rubber pluckers
Evisceration ↓	Viscera removed and carcass washed with inside/outside washer
Chilling / Freezing ↓	Carcasses reduced in temperature by air or water chilling / freezing
Processing & packing ↓	Carcasses packed whole or portioned / skinned (modified gaseous atmosphere may be applied)
Storage / distribution ↓	Finished products stored and distributed at <4°C
Retail storage ↓	
Retail sale ↓	
Consumer	

5.39 Once birds reach the desired weight, they are removed from the house by a catching team that travels to a number of farms to undertake the same activity. Birds are placed into crates and loaded into modules that are transported in vehicles to the abattoir. Most UK farms operate a partial depopulation system where a small proportion of birds (~30%) are removed from the house prior to full depopulation of the remaining birds. This approach is also referred to as thinning which optimises the rearing of birds as they are grown to a population density in the house in line with welfare requirements and therefore removing a proportion once they achieve this density allows the rest to be grown on to larger sizes to occupy the remaining space whilst not exceeding the desired/permitted density. Thinning may occur once or several times depending on the system employed by the farm. As it compromises the biosecurity of the house, thinning is widely recognised as a means of introducing contamination.

5.40 Birds arrive at the abattoir and are stunned prior to slaughter. Stunning occurs by electrical or controlled atmosphere and both are intended to render the bird unconscious prior to killing by severing the carotid artery. Birds, at this point hanging by the feet on shackle lines, are bled and then proceed to processing.

5.41 Processing begins with wetting of the feathers in a scald tank at temperatures of 50-60°C followed by feather removal using rotating rubber 'fingers' that extract the feathers from the carcass without damaging the skin. Following removal of the head and feet, the bird is eviscerated using automated equipment that cuts the vent and mechanically extracts the visceral contents. The bird is washed inside and out with automated equipment and chilled to <4°C or frozen. This is achieved by forced air, water spray or immersion. Chilled carcasses are processed by portioning or de-skinning, mostly undertaken using automated equipment and then packed. Packing may include the application of gases (modified atmospheres) to reduce microbial growth and increase product shelf life. Product is distributed for further processing i.e. cooking, or for retail or catering sale.

Chicken as a source of *Campylobacter* spp.

5.42 In our second report on *Campylobacter* (490) we noted: (i) that *Campylobacter* spp, (principally *C. jejuni* and, to a lesser extent, *C. coli),* were common in

commercial poultry flocks, with approximately 60% of housed broiler flocks being *Campylobacter*-positive at slaughter age and (ii) that there appeared to be a general trend towards lower colonisation rates in the UK. Prior to reviewing the relative prevalence of *Campylobacter* spp. in flocks and on chicken meat it should be noted that the nature of the sample and the method used for the isolation of *Campylobacter* spp. from chicken can significantly influence the detection and enumeration of the organism. Consequently, it can be difficult to compare results from different surveys.

5.43 Based upon predominantly caecal samples across EU Member States, the prevalence of *Campylobacter* colonization in broiler batches was 71% in 2008 (491) although this varied from 2% to 100% between different Member States. Northern European countries (Sweden, Norway, Finland, and Denmark) had markedly lower prevalence than southern member states. By 2014, this figure had decreased to 30.7% (of 13603 units tested) in 20 EU Member States (31.8% of broiler slaughter batches and 30.3% of flocks tested) (489). The prevalence in UK slaughtered batches of chickens was amongst the highest in the EU at 77.9% (from a sample of 426 slaughter batches). In contrast to the reduction in contamination noted above in the EU since 2008, the UK figure was unchanged (75.3% from 401 slaughter batches in 2008).

Table 5.3. *Campylobacter* spp. in broiler chicken in 2014 in the EU (from EFSA, 2015)

Country	Matrix	Description	Source	Sampling unit	Number Tested	Positive	Positive (%)
Austria	broilers	Animal - caecum	Slaughterhouse Official	herd/flock	530	306	57.74
Croatia	broilers	Animal - caecum	Farm Official	herd/flock	918	196	21.35
Czech Republic	broilers	Animal - caecum	Slaughterhouse Official	slaughter batch	281	156	55.52
Denmark	broilers, before slaughter	Animal - cloacal swab	Slaughterhouse Industry	herd/flock	3474	964	27.75
Estonia	broilers	Animal - caecum	Slaughterhouse Official	herd/flock	73	0	0
Finland	broilers	Animal - caecum	Slaughterhouse Industry	slaughter batch	1507	91	6.04
			Slaughterhouse Industry	slaughter batch	341	6	1.76
Germany	broilers	Animal - caecum	Slaughterhouse Official	slaughter batch	637	321	50.39
Greece	broilers	Animal - caecum	Slaughterhouse, Official	herd/flock	494	453	91.7
Italy	broilers	Animal	Farm	animal	4	1	25
Latvia	broilers	Animal - caecum	Slaughterhouse, Official	herd/flock	147	93	63.27
Portugal	broilers	Animal - caecum	Slaughterhouse, Official	herd/flock	681	601	88.25
Slovakia	broilers	Animal - caecum	Slaughterhouse, Official	herd/flock	428	55	12.85
Spain	broilers	Animal - caecum	Slaughterhouse, Official	slaughter batch	500	267	53.4
Sweden	broilers	Animal - caecum	Slaughterhouse, Official	herd/flock	3162	32	1.01
UK	broilers	Animal - caecum	Slaughterhouse, Official	slaughter batch	426	332	77.93

Prevalence of *Campylobacter* spp. on chicken meat

5.44 EFSA (489) reported an average prevalence of 36.7% from 1519 single retail samples of raw broiler meat across 10 EU Member States in 2014. The prevalence in the same report from carcasses sampled post slaughter was 44.4% (3370 single samples). This compares favourably with the prevalence reported in the EU baseline survey from 2008 (491) of 75.8% (post chilling), although the sampling from the latter survey was more standardised and included more countries and the results were therefore likely to be much more representative. The survey in 2008 also assessed the levels of contamination on carcasses and showed significant variation between different member states with the proportion of carcasses with counts below the limit

of detection (<10per g) ranging from 3.8% to 98.6% and the proportion with very high counts (>10000per g) ranged from 0% to 31.9%.

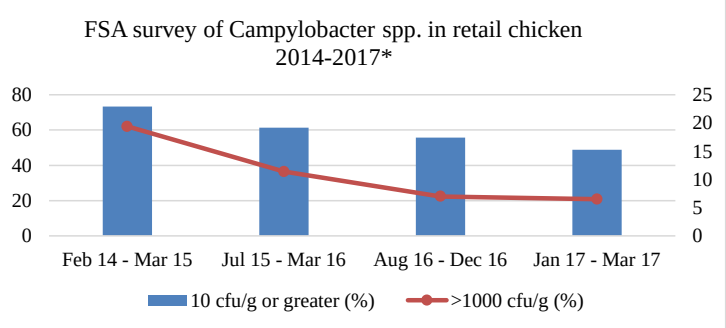
5.45 In the UK, a standardised survey of the prevalence and levels of *Campylobacter* spp. on raw, whole fresh chicken has been conducted since 2014 at both slaughter plant (post chill) and at retail. This was aimed to monitor the FSA/industry strategy to reduce contamination in fresh whole retail chicken with a target set to reduce the most heavily contaminated birds (those with over 1000 cfu/g neck skin) from an estimated 27% in 2008 to below 10% post chill by the end of 2015 (313) and subsequently to <7% at retail. This reduction was estimated to be capable of achieving a reduction in human cases of between 15% and 30% (up to 111000 cases per year) (313, 492).

5.46 A total of 4011 raw, chilled, whole chickens were collected from retail establishments between February 2014 and March 2015 (493). *Campylobacter* spp. were enumerated from samples of neck flap and separately from the outside of the packaging. The prevalence (10 cfu/g skin or greater) was found to be 73.3% and 19.4% had levels above 1000 cfu/g skin. Outer packaging was found to be contaminated on 6.8% of products and 1.6% of samples were found to have levels between 100 and 4500 cfu/swab. Seasonal effects were evident with a higher proportion of chickens having high counts in the summer months and increased weight of bird also increased the likelihood of higher counts. *C. jejuni* was the predominant isolate in the samples (76.6%), whereas *C. coli* was found in 13.9% and both species were present in 4.2% of samples. *C. coli* was more frequently isolated in samples during the summer and in birds with access to the external environment, i.e. free range or organic.

5.47 In the second year of the FSA survey, 2998 fresh retail chickens were sampled between July 2015 and March 2016 (494) the prevalence (10 cfu/g skin or greater) had reduced to 61.3% and to 11.4% for those with the highest counts (>1000 cfu/g skin). Outer pack contamination also reduced to 5.5%. Similar seasonal and weight effects were observed as those noted in the first survey (above) and predominant isolates remained *C. jejuni* (83%) with *C. coli* in 13.5% of samples and both in 3.4% of samples.

5.48 Survey results between August and December 2016 of 1462 chickens found a further reduction in overall prevalence (10 cfu/g skin or greater) to 55.8% with the highest levels of contamination (>1000 cfu/g skin) significantly lower at 7% (495). There was, however, a change in the amount of sample (neck skin) used for the analysis due to an industry wide introduction of neck flap removal to reduce the amount of contamination on the bird. An industry study on the effects of removal of neck flap on the levels of *Campylobacter* spp. through carcase rinse concluded that reducing the neck skin length on a chicken carcase to no more than 5cm did not impact on the risk associated with the level of *Campylobacter* spp. on whole chicken carcases (http://www.campylobacter.org.uk/neck-skin-reduction/). More extensive studies conducted by Jorgensen et al (2016) comparing neck skin of variable quantities with back skin and whole carcase rinse demonstrated that all three methods can enumerate *Campylobacter* spp. from fresh chicken. When estimating contamination at 1000per gram or more, neck skin and whole carcase wash recovered similar numbers of Campylobacters and back skin showed a lower prevalence. The weight of neck skin had some effect on the numbers of *Campylobacter* spp. recovered with 10g or less of neck skin recovering on average – 0.425 $\log_{10}$cfu/g in comparison to 25g of neck/breast skin. When smaller amounts of neck skin were compared there was limited evidence of a difference in levels recovered between weight ranges of 1-5g and 6-10g neck skin. Whilst the differences are not marked it would be our view that the data using 25g of neck skin are not directly comparable with those where 10g or less of neck skin is recovered and it would be best to consider the survey results as discontinuous in this regard.

5.49 Notwithstanding this, using the 10g neck skin protocol the most recent FSA survey of 1051 whole fresh chickens between January and March 2017 has shown an overall contamination level of 48.8% with levels greater than 1000cfu/g at 6.5% (496)



*: Jan 17 – Mar 17 different sampling protocol

Figure 5.6 Prevalence of *Campylobacter* spp. on retail chicken in the UK

Contamination sources in chicken production

5.50 In our second report we identified several sources of contamination and routes of transmission of *Campylobacter* spp. to the chicken on farm and provided a detailed review of each of these.

Sources of contamination identified in ACMSF Second Report on *Campylobacter*

- vertical transmission from parent flocks;
- contaminated water;
- contaminated feed;
- carry-over from a previous flock;
- domestic and/or wild animals and birds;
- the external environment around the broiler house;
- contaminated footwear and clothing of farm personnel and visitors;
- transfer of contaminated equipment between houses.
- contaminated transport crates, vehicles and personnel at flock thinning and when birds are weighed or maintenance is carried out;
- equipment at times other than thinning;
- feed withdrawal

5.51 Since this report other studies have been conducted that further confirm these as potential sources of contamination. Some of these studies have attempted to quantify the relative importance of each of these in the colonisation of the flock. In a

comprehensive review conducted by EFSA (497), a quantitative microbiological risk assessment (QMRA) was undertaken to rank/categorise intervention strategies throughout the farming and production process for chicken. This was based on studies conducted in one or more member state and the key conclusions are summarised below. It should be noted that as some of these factors were based on studies in a single member state the interventions may not have the same impact in primary production systems in other states. Key conclusions:

- Vertical transmission does not appear to be an important risk factor;
- Biosecurity measures are essential to prevent flock colonisation;
- Colonization with *Campylobacter* of flocks with outdoor access is very likely to occur;
- Fly screens effectively reduces flock colonization in summer and thereby reduces public health risk by 50 to 90%;
- Thinning (partial depopulation) is a risk factor for flock colonization and stopping this practice is estimated to reduce the risk by up to 25%;
- Restricting the broiler slaughter age of indoor flocks to 35 or 28 days would reduce the public health risk by 10 to 20% or up to 50%, respectively;
- Reducing the numbers of *Campylobacter* in the intestines at slaughter by 3 log10-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;
- 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 colonised flocks by at least 3 log10-units;
- 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.

5.52 In general, this reinforces many of the points identified in our Second Report and together with more recent published research and systematic reviews, the following points can be concluded.

Biosecurity measures;

Vertical transmission

5.53 This remains a potential source of *Campylobacter* spp. into poultry houses either by direct vertical transfer to chicks or by so-called pseudo-vertical transfer i.e. infection of chicks through contamination of the egg. Agunos et al. (498) noted in a systematic review that there was some evidence of vertical or pseudo-vertical transmission of *Campylobacter* spp. although the relative importance of this could not be determined. In another systematic review, Newell et al. (499) similarly concluded that vertical or pseudo-vertical (contaminated eggs, fluff, etc.) transmission was possible but not considered to be a major contributor to flock infection. However, Cox et al (500) reviewed the evidence for horizontal and vertical transmission from hen to chick and considered this to present a significant source of infection to chicks in the farm estimating that egg transmission could be as high as 1 in 133 to 1 in 13333 eggs. They cited the lack of sensitive sampling and detection methods for the reason why such low initial levels of infection do not evidently appear widespread in the flock for several weeks. Other studies have failed to recover *Campylobacter* spp. from chicks hatched under laboratory conditions and maintained under high levels of biosecurity for the purposes of use as controls in experimental studies (501).

5.54 *Vertical or pseudo-vertical transmission is not considered the most significant source of contamination in the chicken house, but there is sufficient evidence to indicate that this can occur and it is recommended that it is not dismissed as a potentially important area for control.*

Poultry house including cleaning and disinfection

5.55 Agunos et al (498) identified the highest risk practice for contaminating a flock with *Campylobacter* spp. to be a contaminated barn environment caused by inadequate cleaning and disinfection together with restocking too quickly and the presence of adjacent broiler flocks. Shreeve et al (502) studied the molecular

patterns of isolates from a number of successive broiler flocks and found the identical strains in 16% of the 60 broiler houses studied. In contrast, carryover from one flock to another due to inadequate cleaning and disinfection was not considered as significant factor in the review by Newell et al (499). Battersby et al (503) evaluated cleaning practices in 20 houses from 10 farms and found *Campylobacter* spp. in a number of sampling points following cleaning and disinfection including the tarmac apron, ante-room, house door, feeders, drinkers, walls, columns, barriers and/or bird weighs. They also evaluated several disinfection regimes for their efficacy and recommended farms review their practices to prevent cross-contamination from successive flocks. Newell et al (499) did not find house age or the number of houses on the same site to be a significant risk factor associated with flock infection. Smith et al (475) reported that two or more houses on a farm were associated with reduced odds of *Campylobacter* spp. colonisation (low performing farms). Sommer et al (504) however, reported that in a 2-year study of nearly 6000 broiler flocks in Denmark having more than one house on a farm was significantly associated with the presence of *Campylobacter* spp. in the flock.

5.56 *Carryover from successive flocks due to inadequate cleaning and disinfection between them is a potential risk and prevention of this should remain a key control in reducing colonisation of flocks. The number of houses on a farm does not appear to be a reliable indicator of risk regarding flock colonisation by* Campylobacter spp.

Water and feed

5.57 Feed has always been a potential source of contamination into the poultry house (505) but the large scale commercial production of feed means that this is now considered to present limited risk of contamination and has not been identified as a significant risk factor in recent scientific reviews. However, the potential for feed systems to act as a vehicle to spread contamination in the house may be of more significance as they are often found to be contaminated with *Campylobacter* spp. (503, 506). The management of feed storage on the farm is clearly an additional risk and the general principles regarding management of pests equally apply to feed.

5.58 Water is frequently cited as a significant potential risk for contamination of flocks on farm including water in the farm environment, water supplied to birds to

drink and water systems that the bird drinks from (498, 499). Water in the external environment i.e. puddles, rivers, etc. can contain *Campylobacter* spp. and provide a source of contamination if introduced into the house either through poor biosecurity i.e. from boots walking through puddles or as a drinking water source i.e. untreated river water. Water and feeding equipment in the house are recognised as significant potential vehicles to spread *Campylobacter* spp. due to potential fouling by birds (506).

5.59 Ogden et al (507) examined the presence of *Campylobacter* spp. in a variety of water sources and equipment in a number of Scottish poultry houses and then used MLST to type strains. They recovered the organism from water, independent of the source or whether treated or untreated. The same *Campylobacter* spp. MLST type was found in the header tank and from birds in the same house on 1 of the 12 farms although it was not clear which direction the contamination occurred i.e. header tank to bird or *vice versa*. Battersby et al (503) found *Campylobacter* spp. In water and feeding systems after cleaning and disinfection. In the systematic review conducted by Agunos et al (498) there was only one study that identified a significant association between flock colonisation and water, in this case untreated water. Similarly, Newell et al (499) considered evidence indicating an association between drinking water and flock infection to be largely circumstantial and highlighted studies where chlorination of drinking water was applied that showed both statistically significant reductions in flock colonisation and others that showed no statistical reduction. Klein et al (508) recently reviewed research on the use of disinfectants in poultry drinking water on the reduction of caecal contamination. Results were generally favourable using organic acids (lactic, propionic, acetic, etc), caprylic acids and electrolysed water but the effects at an industrial scale was reported to be variable.

5.60 *Feed and water are both potential sources of contamination into the poultry house but in well managed commercial broiler houses they are not considered a major source of incoming contamination, although untreated water sources may be considered of higher risk and the potential for water tanks and feed and water systems in the house to harbour and spread contamination may be significant and should be effectively managed especially during cleaning and disinfection cycles.*

Litter

5.61 Litter was not considered a significant risk factor in the systematic reviews by Agunos et al (498) and Newell et al (499) although once litter becomes wet the risk of infection of the birds was reported in one study to double. Smith et al (509) studied the survival of *Campylobacter* spp. in poultry faeces and litter under different environmental conditions. Survival was significantly longer in faeces than in litter. Humidity (<70% and >85%) was not shown to have any significant effect on the survival of *Campylobacter* spp. whereas temperature did, with storage at 20°C significantly enhancing survival than storage at 25°C or 30°C. Line (2006) studied the effect of humidity on the transmission of *C. jejuni* in broiler chickens and a significant difference was seen in colonization rates between low (30% RH) and high (80% RH) relative humidity conditions with a delay being observed in chickens grown under low humidity conditions.

5.62 This has been recognised by industry and as part of some processors farm interventions they have introduced biomass boilers to heat the house to reduce the amount of moisture entering. Gas fired heaters are reported to introduce much greater moisture into the shed and consequently biomass boilers result in reduced litter wetness, reduced *Campylobacter* spp. colonisation and improved welfare (personal communication).

5.63 *Given the practical success of reduced litter moisture in reducing* Campylobacter *spp. colonisation, it is recommended that this is further explored as a potential on farm intervention.*

Flies

5.64 *Campylobacter* spp. have been isolated from a wide variety of animals including other farm species (510), birds (511) and insects including flies (512, 513). Hald et al (512) conducted an 8-month study collecting flies from poultry farm surroundings in Norway and found 31 / 2816 (0.01%) positive by enrichment for *Campylobacter* spp. with the prevalence highest on a mixed pig-poultry farm (up to 28%). The same study used insect trapping to estimate an average incursion per broiler flock rotation of 30728 insects of which 21.4% were flies. In a similar study in

the UK, Royden et al (513) reported an individual fly prevalence of 0.22% (2 / 902) and a composite prevalence of 0.01% (127 samples of 1293 pooled flies). The isolates were shown by MLST to be broiler-associated types responsible for human illness. Hald et al (514) demonstrated a reduced *Campylobacter* spp. flock prevalence rate from 51.4% in control flocks to 15.4% in those where fly nets were used.

5.65 Flies entering a broiler house appear to present a potential risk of introducing Campylobacter spp. and fly nets have been successfully employed in Scandinavia to reduce the rate of flock colonisation. This may be associated with other risk factors such as contamination in the environment and the risk may fluctuate and increase in the summer, but it is recommended that the control of insects, and flies in particular, receive further investigation.

Depopulation

5.66 Another factor that has been reported in many studies to increase the risk of flock positivity is thinning (partial depopulation). In the systematic review conducted by Newell et al (499) the risk of flock positivity was reported to increase when thinning crews were large, not farm dedicated or poorly educated. The time between thinning and full depopulation was considered important in the context of within-flock prevalence with the principle that the shorter this time the less opportunity for maximum flock colonisation.

5.67 Allen et al (515) studied the sources and spread of *Campylobacter* spp. during partial depopulation of 51 flocks over 2 years sampling environmental, house litter / faeces and caecal samples. They reported that at partial depopulation 21 flocks were positive for *Campylobacter* spp. and all caecal samples were positive with mean levels of 8 $\log_{10}$ cfu/g. A further 27 flocks became positive within 2 to 6 days of partial depopulation and had similarly high levels of caecal colonisation. Prior to thinning *Campylobacter* spp. were found extensively in the environment (farm driveways, transport vehicles, equipment and personnel) and PFGE found similar types in specific sampling areas and those isolated from thinned flocks.

5.68 Lawes et al (516) conducted a 3 year stratified randomized survey of UK chicken broiler flocks to determine the prevalence of the *Campylobacter* spp. at slaughter and the associated risk factors. Slaughter batches (1174) were found to be contaminated at a rate of 79.2% from the 37 abattoirs included in the study and previous partial depopulation was found to be statistically associated with flock prevalence (odds ratio (OR) 5·21).

5.69 Smith et al (475) reported on a year long study of the impact of biosecurity and partial depopulation on *Campylobacter* spp. prevalence in Irish broiler flocks with differing levels of performance (economic, hygiene and biosecurity). Although farms with high performance had significantly lower prevalence of *Campylobacter* spp. at first depopulation than lower performance farms across all seasons these differences were not observed at final depopulation when prevalence was over 85% in both high and low performance farms.

5.70 Georgiev et al (517) assessed the impact of enhanced biosecurity measures on farms on the prevalence of *Campylobacter* spp. from 2314 poultry batches sampled at slaughter in the UK between 2011 and 2013. Enhanced biosecurity reduced the likelihood of colonization at partial depopulation [odds ratio (OR) 0·25, 95% confidence interval (CI) 0·14-0·47] and, to a lesser extent, at final depopulation (OR 0·47, 95% CI 0·25-0·89). The authors calculated that approximately 1/3 of highly colonised batches (>123000 cfu/g in pooled caecal samples) could be avoided if they were all raised under enhanced biosecurity or without partial depopulation and that such on farm measures could play a role in reducing human exposure.

5.71 Extensive studies conducted by the UK poultry industry have demonstrated that prolonged periods without thinning together with high standards of biosecurity can reduce the colonisation rate on farms, although this is more challenging in warmer months and does not deliver a consistent absence of flock infection (518). These studies compared model farms with standard ones and sampled caeca and neck skin samples from 883 crops in 16 farms over 2 years and demonstrated that stopping thinning could result in a reduction by 12% of *Campylobacter* positive flocks. The same study also demonstrated that reducing the period between first thin and total house depopulation can significantly reduce the extent of colonisation of

the flock and therefore the amount of *Campylobacter* spp. entering the processing plant.

5.72 *Evidence clearly demonstrates that partial depopulation is associated with subsequent* Campylobacter *spp. colonisation of the flock. Whilst avoiding thinning may be a better theoretical approach to reducing colonisation of flocks, the application of phased thinning (reduced time between thinning and full depopulation) may be a more practical and commercially viable option, especially during certain times of the year when the* Campylobacter *spp. colonisation risk is higher i.e. summer. It is recommended that industry continues to investigate this option.*

Human activity

5.73 Human traffic associated with farm staff incursions into a house was reported to be related directly to an increased risk of flock colonisation particularly when people handle other livestock and poultry (499). This is not surprising given the evidence of extensive contamination of the farm environment with *Campylobacter* spp (519). Consequently, enhanced biosecurity has been shown to be associated statistically with reduced rates of flock colonisation (475, 517). Newell et al (499) reported that measures to prevent entry and spread of *Campylobacter* spp. into a house such as house-specific boots and clothes, overshoes and the effective use of boot dips were all associated with a reduced risk of flock infection. In addition, the introduction of effective hygiene barriers in the entrance of the house that separate the dirty side from the clean side have been shown to reduce the risk of flock infection by approximately 50%.

5.74 There has been a major improvement in the introduction of hygiene barriers throughout the UK farm population of the major poultry processors, with many adopting double entry systems that allow external footwear to be removed in one area, stepping into a neutral zone and then applying new footwear and outer clothing in the final ‘clean’ zone.

5.75 The ability to maintain biosecurity measures can be difficult for farm workers particularly when competing priorities and pressures exist. Millman et al (520) assessed the biosecurity awareness, interpretation and practice amongst poultry

catchers and identified that whilst awareness of biosecurity measures was generally good and enhanced by training the key factors impacting on lapses in biosecurity were time pressure and lack of suitably hygienic equipment. Use of financial incentives for farmers to adopt high levels of biosecurity and therefore lower prevalence of on-farm *Campylobacter* spp. has been reported to have a positive effect (521).

5.76 *It is recommended that the practice of introducing hygiene barriers is adopted throughout the industry to reduce the number of flocks colonised by* Campylobacter *spp. Further measures to reduce the risk of contamination of flocks by contaminated catching equipment and catchers need to be explored.*

Bird associated factors

5.77 Johnson et al (522) reviewed treatments with potential to reduce *Campylobacter* spp. colonisation of poultry including probiotics, prebiotics, bacteriophage, bacteriocins, vaccines and small molecule inhibitors.

Small molecule inhibitors

5.78 Johnson et al (523) and Kumar et al (524) reported on studies assessing the impact of small molecule inhibitors on the growth and survival of *Campylobacter* spp. Molecules referred to as 'campynexins' were found to variously inhibit flagellar motility, *in vitro* growth and also induced intracellular clearance from human intestinal Caco-2 cells. Studies in day old chicks by Johnson et al (523) demonstrated significant reduction in *in vivo* caecal colonisation of *Campylobacter* spp. but results were inconsistent.

Vaccination

5.79 Development of effective vaccines to protect against *Campylobacter* spp. colonisation in chickens remains problematic. Several researchers have explored a variety of different antigens and their efficacy against *Campylobacter* spp. *in vivo*. Johnson et al (522) summarised the different antigens used by these researchers to develop potential vaccines that have variously included total outer membrane proteins, fusion proteins, adherence and colonisation proteins and periplasmic proteins and concluded that the most effective candidate antigens were surface-

located proteins as they are more likely to be more accessible to illicit an immune response in the host. Neal-McKinney et al (525) demonstrated a 3 log_{10} cfu/g reduction in *C. jejuni* isolated from caecal samples in chicks after oral challenge and following injection with the recombinant fusion protein (CadF-FlaA-FlpA). This was greater than that observed using the individual surface exposed colonisation proteins although both FlpA and FlaA also achieved significant reduction in caecal counts. Antibody responses were also assessed and whilst control non-vaccinated birds were shown to have a low level of anti-*Campylobacter* antibodies, the greatest antibody response was elicited by FlaA vaccination. Although effective, the administration of vaccine by injection would preclude its use operationally but the potential to incorporate such antigens into surrogate hosts in feed could offer some potential. As demonstrated in this research, vaccines have been reported to achieve reductions in caecal contents between 1 and 6 log_{10}/g but the effects are somewhat variable (526, 527)

5.80 *The development of a vaccine against* Salmonella *Enteritidis was a major factor in the control of the organism in chicken and therefore human infection caused by eggs and whilst the prospect for similar effective vaccines for* Campylobacter *spp. remain more elusive, continued research is progressing and may in the future provide for an effective control.*

Competitive exclusion

5.81 The use of bacterial cultures administered to chicks in order to promote the development of a microbiota that can reduce colonisation with *Campylobacter* spp. through competition for adherence sites, production of inhibitory compounds or by other means not yet understood has been evaluated and applied in commercial poultry farms for many years with varying reported levels of success (528). The principles and application of probiotics has been reviewed recently by Perumalla et al (529). Commercially available products include those with defined microbial strains derived from caeca (Broilact, Aviguard) or with undefined strains (Mucosal starter culture). Many of the products were developed principally for control of infection with other enteropathogens including *Salmonella* spp. and *Clostridium* spp. but have subsequently been applied for the control of *Campylobacter* spp. Schneitz and Hakkinen (530) studied the effect of one commercially available product

(Broilact) on the colonisation of chicks given the CE product on the day of hatch. Seeder birds (previously infected with *Campylobacter* spp.) were then added to the chicks that had been given CE product and a control group that did not. The results demonstrated a significant reduction in the rate of colonisation in the treated chicks (0% and 30% at week 1 and 2, respectively in comparison to 100% in the control chicks) although by week 4 and 5, 95% and 90%, respectively of the treated chicks were colonised. The levels of *Campylobacter* spp. in the caecal contents was 1.4 logs lower at the end of rearing in the treated chicks demonstrating some significant benefit in the use of the CE product. Studies using individual cultures have shown the significant inhibitory effect conferred by strains of *Lactobacillus* spp. on *Campylobacter* spp. (531, 532).

5.82 *Studies on the microbiome and its impact on the health of animals and humans has been gathering pace in recent years and whilst the effects of competitive exclusion techniques have been somewhat studied in a cause and effect manner, it is recommended that such approaches are encouraged to identify key factors in the chicken microbiome that may prevent colonization with* Campylobacter *spp.*

Bacteriophage

5.83 Recent reviews of the potential for bacteriophage to reduce levels of *Campylobacter* spp. in chicken intestine and also on the surface of chickens have been published by Lin (533), Connerton et al (534), Klein et al.(508) and Johnson et al (522). Bacteriophage are viruses that selectively infect and destroy bacteria. They can be highly specific in their target host and due to this trait have in the past been used for typing of bacteria. Bacteriophage infect the host cell, multiply and depending on the type can lyse the cell to release large numbers of copies that can then subsequently infect additional cells. As they utilise the functioning host cell to replicate, they are most effective when the host is actively metabolising which makes bacteriophage treatment of particular interest for intestinal reduction of *Campylobacter* spp. in chicken. Connerton et al (534) summarised the studies reporting prevalence of 3-20% in poultry samples with the levels of *Campylobacter* spp. in birds where *Campylobacter* specific bacteriophage were naturally present being on average 1.8 $\log_{10}$cfu/g lower than birds where they were not found. Studies

exploring the effect of bacteriophage on the levels of *Campylobacter* spp. in chicken intestinal/caecal samples were reviewed by Johnson (522), Klein et al (508) and Connerton et al (534). These demonstrated reductions of 1.5-5.6 log_{10} cfu/g in comparison to non-treated controls. Although further work is needed to explore the potential impacts on selecting strains with good survival characteristics through the chicken gut and broad-spectrum *Campylobacter* spp. efficacy, there does seem to be some prospect that bacteriophage could play a role in the control of this organism. Studies have also been conducted on the application of bacteriophage to the chicken skin after processing and whilst this suffers from the host not being in a particular active metabolic state (as it will not multiply at chill temperatures) there is some evidence of an effect in disrupting the cell wall due to physical attachment and invasion. Atterbury et al (535) reported a 1.1-1.3 log_{10}cfu/g reduction in *Campylobacter jejuni* after treatment with phage and storage at 4°C and similar reductions (1.25 log_{10}cfu/g) were also reported by Goode et al (536).

5.84 The development of viruses with sufficient coverage of all pathogenic *Campylobacter* spp. and the potential issues associated with resistance have been raised as potential problems with the use of bacteriophage although the use as a terminal inactivator would certainly serve to address the latter point.

5.85 Bacteriophage treatment of chickens to reduce infection in the gut or as a post-process addition for surface decontamination shows some potential for reducing levels of *Campylobacter* spp. in/on chicken and more in vivo studies examining the potential for this technology and a cost benefit analysis may be useful in establishing it as a proper contender for intervention. Its legal status will also need to be considered.

Bacteriocins

5.86 Bacteriocins are peptides produced by bacteria that have an antimicrobial effect on other bacteria which may be broad or narrow spectrum. The most famous of these is probably nisin that is produced by *Lactococcus lactis* and is used for the control of *Listeria monocytogenes* in some foods. Several bacteriocins have been isolated that have been shown to be effective in reducing *Campylobacter* spp.

Lin (533) has reviewed comprehensively the studies on anti-*Campylobacter* bacteriocins and this is further reviewed by Johnson et al (522) Bacteriocins SRCAM 602, OR-7, E-760 and E 50-52 administered in feed or drinking water were all shown to reduce *in vivo* levels of *Campylobacter* spp. by 2.2->6$\log_{10}$ cfu/g in chickens, providing significant evidence of their potential as an intervention. However, whilst bacteriocins do offer some potential there remain several issues that need to be addressed before they could be considered for use including the commercial viability, development of resistance, effect on other chicken intestinal microflora and safety assessment on humans.

5.87 Bacteriocins have demonstrable benefit in reducing *Campylobacter* spp. colonisation of chicken intestines but significant work still remains to be done to make this intervention viable for use in the near future. However, this should not preclude the continued study and development of this approach.

Feed conversion rates

5.88 Smith et al (475) reported that improved economic performance and feed conversion rates were associated with high performance farms that had 20-40% lower *Campylobacter* spp. prevalence at first thin in comparison to low performance farms. Higher recent mortality has also been reported to be associated with increased risk of flock colonisation (516). The impact of bird colonisation on bird health is considered in Section 6.

Catching and Transportation

5.89 Once grown to a desired weight, birds are removed from the houses by a catching team. This is usually conducted with modules that contain plastic or metal drawers (crates) into which the chickens are placed. The modules/crates are driven by forklift into the house and, once full, are loaded onto transport vehicles. This stage of the process is recognised as being a significant potential vehicle for the introduction of *Campylobacter* spp. into the house and consequent colonisation and spread of the organism amongst the flock. Equipment used by catching crews is used between houses and on different farms and without dedicated equipment it is clearly not possible to exclude the organism that will be present in the surrounding environment. This is less significant for sheds that are fully depopulated but for

partially depopulated/thinned flocks the vehicles, equipment, personnel and general breaching of biosecurity introduces contamination (see depopulation section) (537). Some work has been conducted to improve the biosecurity of catching through training of catchers, disinfection of vehicles, use of conveyor belt catching systems to preclude the need for a vehicle to enter the shed, but these have not proven to be capable of excluding the organism completely.

5.90 Crates and transport modules have also been frequently identified as vehicles for spread of contamination as birds defecate during transport and an accumulation of faeces is evident on both of these units. Spread of contamination can therefore occur to birds in the same crate or in other crates due to contact with contaminated faeces and whilst the transport duration is generally too short to result in internal colonization of the bird, external contamination on the feathers can be extensive. In addition to the direct contamination of birds during transportation, the crates and modules present a significant vehicle for contamination of subsequent flocks when they are reused for catching. Slader et al. (537) demonstrated a high prevalence of *Campylobacter* spp. on unwashed and washed crates and whilst numbers were reduced by the use of detergent and disinfectant, it was not possible to completely eliminate the organism. Hansson et al (538) reported 57% contamination of cleaned and disinfected crates with *Campylobacter* spp. and genetic subtyping demonstrated contamination of chickens during transport to slaughter. Poultry processors usually have automated systems for the cleaning and disinfection of both crates and modules but the effectiveness in reducing or eliminating the organism is highly variable depending on the type and optimisation of the cleaning units. One of the key challenges is removal of the large amount of dried faeces on the crates which have difficult to access areas and it is not uncommon to evidence ‘clean’ crates with residual faecal contamination.

5.91 Equipment used for catching and transportation of birds from farm to the processing unit present significant vehicles for the transfer of contamination to birds within the same flock and to subsequent flocks and further development of improved systems of capture and transport to reduce spread of the organism are encouraged.

Processing

5.92 Once received at the slaughterhouse chickens are killed, de-feathered, eviscerated, rinsed, chilled (or frozen) and then further processed. These stages spread *Campylobacter* spp. extensively over the bird and across the batch (539) but many opportunities exist to reduce the spread of contamination and, indeed, in recent years the introduction of in-line process interventions has led to significant reduction in contamination of chickens in the UK.

5.93 In the assessment published by EFSA (453) quantifying the risk in chicken production, the following conclusions were drawn regarding slaughter associated risks;

- 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 than 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 was prevented;
- scheduled slaughter aims to identify colonised 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 colonised 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 colonised 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.

5.94 Risk factors in the slaughter and processing of chickens associated with the contamination levels of broiler carcasses with *Campylobacter* spp. were reported by Seliviorstow et al (540) as being; the contamination level (internal and external) of the incoming birds, the duration of transport and holding times (linked to feed withdrawal), the unloading system, electrical stunning, lower scald temperature, incorrect setting of the plucker, vent cutter and evisceration machines. Pacholewicz et al (541) studied the contamination levels of *Campylobacter* spp. and *E. coli* during processing and concluded that bacterial concentrations in the caeca and excreta were the most prominent of 19 variables on the concentration of the organisms on carcases. Rosenquist et al (542) reported a correlation between *Campylobacter* spp. concentrations in the intestinal contents and on the chicken carcasses after defeathering and concluded that a reduction of levels on the carcass may be achieved by interventions that reduced the concentration in the intestines of living birds. Allen et al (543) also showed that contamination of carcasses from negative farm flocks were much lower (</=30%) than those from partly colonised flocks (90-100%) and those from fully colonised flocks (100%) and that they had statistically higher counts per carcass (average 5.3$\log_{10}$cfu) than low prevalence flocks (average

2.3log$_{10}$cfu). They also reported the finding of high numbers of *Campylobacter* spp. in aerosols, particles, and droplets in the hanging-on, de-feathering and evisceration areas but not the chiller. Indeed, they reported a significant reduction in numbers on carcasses before and after forced air-chilling and also between plucking and chilling. Although current processing has the general effect of spreading contamination from the bird faeces to exposed surfaces, it is recognised by the industry that optimisation of these stages can achieve a significant reduction in spread of contamination and the levels found on the final carcase/ portions. Scald tank temperature, evisceration efficiency, inside-outside washer optimisation can all assist in reducing the general loading; however, with the exception of inside-outside washing (544), they do not achieve a significant reduction in contamination and further process reductions need to be applied to achieve this goal.

5.95 The optimisation of current processing equipment in the production plant to minimise spread of contamination e.g. plucking and to reduce contamination e.g. inside outside washing can significantly reduce the spread of *Campylobacter* spp. from the intestine onto the surfaces of the chicken as the industry is encouraged to continue to focus on these areas.

Chemical treatment

5.96 Treatment of poultry with chemical washes is not allowed in the EU although a number of technologies have been demonstrated to achieve reductions in levels of *Campylobacter* spp. on carcasses. (545)(2007) studied the effect of a number of chemical treatments on levels of *Campylobacter* spp. on chicken carcasses and reported that reductions of 1-2log$_{10}$cfu were achieved, with the most effective treatments being acidified sodium chlorite, trisodium phosphate followed by peracetic acid and chlorine dioxide which was the least effective. Longer treatment times of 30s were most effective. (546) studied several chemical treatments including lactic acid sprays, ozonated water and cold plasma to reduce *Campylobacter* spp. on chicken. They reported that the most effective lactic acid treatment was a 21s dose of 8% acid which resulted in a 1.9log$_{10}$cfu reduction on breast skin but this altered its appearance. However, a 7s treatment of 4% acid gave a 0.8log$_{10}$cfu reduction in levels of the organism. Ozonated water and cold plasma had no effect in reducing *Campylobacter* spp. Zakariene et al (547) treated artificially inoculated chicken

breast with lactic acid solutions (3% and 5%) for 2 minutes and reported a 1.22log$_{10}$cfu/g and 0.9log$_{10}$cfu/g reduction in *C. jejuni* respectively. Tandrup, Riedel et al (2009) reported a 1.69log$_{10}$cfu reduction in *C. jejuni* after a 1-minute immersion in 2.5% lactic acid although the reduction in sterile water control was 0.95log$_{10}$cfu. Chen et al (548) reported that peracetic acid (0.07% and 0.1%) rinse for 23s achieved reductions of approximately 1.5log$_{10}$ on chicken breast / thigh whereas chlorine (0.003%) was the least effective.

5.97 Chemical treatment of chicken carcasses can reduce the levels of Campylobacter spp. although in general by a relatively low amount (1-2log$_{10}$cfu/g). Currently such treatments are not permitted in the EU and there is also likely to be significant consumer objection to the use of chemicals as a terminal disinfectant. The consumer opinion on the use of chemicals could be usefully gathered in advance of considering any further activity on these technologies.

Neckflap removal

5.98 In recent years the major processors of chicken in the UK have introduced a regime of removing a major proportion of the neck skin. Neck skin has traditionally been considered to represent the most heavily contaminated part of the bird, as it travels upside down on the shackle line and contamination therefore flows down the bird and to the neck skin. Therefore, removal of the neck flap should reduce the overall burden of contamination exposed to the consumer during handling of raw poultry. Limited studies have been conducted on the effectiveness of this approach, although industry studies have reported no statistically significant reduction in contamination levels when comparing a large number of birds with normal and with reduced (5cm) neck flaps using whole carcase rinse technique (549).

Low temperature treatment

5.99 Chilling and freezing have both been shown to have a major effect in reducing *Campylobacter* spp. on chicken carcasses. Freezing has been used as an effective intervention technique in some countries in order to reduce contamination in colonised flocks (550). The impact of freezing is believed to be partly due to the development of ice crystals that cause physical damage to the cell and *Campylobacter* spp. appear to be particularly susceptible to freeze damage.

Freezing is clearly not an option for chilled birds but in recent years there has been significant development in technologies that freeze the outer surface of the skin of the bird rapidly to take advantage of the fact that much of the contamination is on the surface. Crust freezing operates by passing the bird through a tunnel of chilled air, nitrogen or carbon dioxide at temperatures ranging from -5^{o}C to close to -20^{o}C. EU rules preclude the freezing of the muscle and therefore the temperature and duration have to be precisely controlled to freeze the outer surface without freezing the muscle and therefore muscle surface temperatures are designed to decrease to no lower than c.a.-1^{o}C.

5.100 A number of commercial crust freezing technologies exist that claim to deliver approximately a 1log$_{10}$cfu reduction in *Campylobacter* spp. and some have been introduced by large poultry processors. The key disadvantage of these units is the length of the freezing tunnel that occupies significant space in the processing plant and may be difficult to retrofit without significant cost. Few published studies have been conducted on crust freezing. (551) used carbon dioxide crust freezing at temperatures of -55^{o}C to achieve a surface temperature of -1^{o}C and this was reported to deliver a 0.42log$_{10}$ reduction in *Campylobacter* spp. Burfoot et al (546) conducted some benchtop trials using liquid nitrogen sprayed onto chicken carcases in either a chamber or tunnel for 20s and 40s respectively and this resulted in a reduction of 1log$_{10}$cfu or greater.

Thermal processing

5.101 Heat processing has long been recognised as a means to achieve significant reductions in the surface contamination of *Campylobacter* spp. on chicken carcasses. Two approaches have been studied over the years; hot water and steam treatment.

Hot water

5.102 Purnell et al (37) developed a shackle line process to immerse carcasses and demonstrated a significant reduction (>1log$_{10}$cfu) using water at 75^{o}C for 30s or 70^{o}C for 40s although the former led to unacceptable levels of skin tearing during subsequent processing. Corry et al (545) undertook further experiments on artificially contaminated carcasses and was able to achieve a 1.66log$_{10}$cfu/cm^{2} using 75^{o}C for

30s. Hot water immersion treatment can adversely affect the visual quality of the bird due to thermal damage of the epidermis and has been cited as a significant factor deterring the adoption of the technology. However, in recent years a number of major poultry production companies have optimised the process and installed purpose-built hot water treatment regimens either post defeathering or post evisceration and have reported reductions of contamination of approximately 1-2$\log_{10}$cfu/g neckflap (personal communication).

Steam

5.103 The use of steam processing has also been shown to significantly reduce carcass contamination although in early experiments the damage to the epidermis was considered too significant to be commercially viable (552). In experiments conducted by James et al (552) they demonstrated a 1.8, 2.6 and 3.3 $\log_{10}$ cfu/cm^{-2} reduction by exposing the carcass to steam in a chamber for 10, 12 and 20s respectively. Whyte et al (38) reported statistically insignificant reductions in *Campylobacter* when treated with steam at 90^0C for 12s and whilst treatment for 24s resulted in significant reduction (1.3$\log_{10}$cfu/g), visible damage was reported to the outer epidermis. Although the prospect for the use of steam has been somewhat doubtful, given the damage to the skin of the bird and thus the effect on visual quality, recent commercial developments have combined rapid steam treatment with ultrasound to achieve significant reductions in *Campylobacter* spp. on carcasses. A commercial process known as Sonasteam has been shown to deliver an 80% reduction in *Campylobacter* spp. on the skin of chickens using a 1.5s residence time and it is understood that this reduction is significantly increased (>1$\log_{10}$ cfu/g) when initial contamination levels are higher (personal communication). Boysen and Rosenquist (551) evaluated the prototype Sonosteam principle and demonstrated a mean reduction of >=2.51$\log_{10}$cfu/carcass. Musavian et al (553) conducted trials with the Sonosteam unit in a commercial poultry processing plant before the inside-outside washer and found a statistically significant reduction (0.95$\log_{10}$ cfu) in the counts of *Campylobacter* spp. on the breast skin and air chilling applied after Sonosteam achieved a further reduction of (0.35$\log_{10}$cfu). Units of this nature have been installed in some commercial poultry processing plants post evisceration to reduce *Campylobacter* spp. (554).

Commented [MU3]: Word is now spelt correctly to reflect Moy Park's consultation comments.

5.104 All thermal processes, whether hot or cold, require major investment (each unit will cost >£100 000) have high operating costs (energy and line speed) and generally have some negative impact on quality (slight epidermal damage).

5.105 Thermal processing (hot water, steam or crust freezing) delivers a relatively small reduction (1-2$\log_{10}$cfu/g) in levels of *Campylobacter* spp. but it has emerged as the most favoured technology for major poultry processors to introduce and this has now become commonplace as an intervention. The significant investment by the industry in these technologies should be welcomed although it should be encouraged to examine opportunities to achieve further reductions by enhancing the technology further or supplementing it with other interventions.

Vacuum / modified atmosphere packing

5.106 It is generally recognised that levels of *Campylobacter* spp. decrease after packing and during the chilled shelf life of the bird. This has led to the prospect of identifying the factors that are causing the death of the organism such that they could be selectively enhanced.

5.107 Al-Qadiri et al (555) studied the survival of *Campylobacter jejuni* inoculated onto beef and stored at 22°C for 5 days and 7°C for 14 days under three different oxygen conditions (<0.5%; 6-8% and 20%) and reported the greatest reductions (1-2$\log_{10}$cfu) at both temperatures when stored at the lowest and highest oxygen concentrations. Slight decreases (<1$\log_{10}$cfu) were also seen under 6-8% oxygen.

5.108 Rajkovic et al (556) found a 1.5$\log_{10}$ reduction in naturally contaminated chicken carcasses when stored in a gas mixture consisting of 80% oxygen / 20% nitrogen but reported no decrease when carbon dioxide was used in place of oxygen. The reduction was enhanced when the chicken was previously treated with a lactic acid / sodium lactate solution (10% w/v).

5.109 The reduction in *Campylobacter* spp. after packaging and during storage could usefully be studied at a more fundamental level to elucidate the inherent factors contributing to these effects which could then be potentially enhanced

through the process. It is recommended that fundamental research is encouraged in this aspect of *Campylobacter* physiology.

Packaging

5.110 Notwithstanding the objective to reduce contamination arising from farm or processing, it is likely that *Campylobacter* spp. will remain a significant hazard on chicken meat. Most retail chicken is sold in pre-packed containers sealed in a barrier film. This may be top sealed containers or flow wrapped (usually for portions) or shrink wrapped (for whole birds). The objective of packaging is to protect the product from damage and contamination or, in the case of chicken, to prevent it from contaminating the consumer. Major improvements have been made in pack seal integrity in recent years with the use of thicker film allowing a virtual hermetic seal to be applied. The addition of drip pads incorporated into packs has made the current products virtually leak proof although damage to the packaging during transport, display and storage will preclude these controls. This is an important advance from previous years where chicken was wrapped in thin packaging without heat sealing and where leakage occurred onto shelves and in customer shopping bags. External surfaces of the packaging can be contaminated with low levels of *Campylobacter* spp. as demonstrated in the surveys of retail chicken discussed earlier in this chapter and the major processors of chicken have invested in processes and in some cases equipment such as ultra violet light tunnels to reduce such contamination further.

5.111 A further step taken by many retailers has been to introduce ‘cook in bag’ chickens that preclude the need to handle the exposed bird and therefore prevent the significant potential cross contamination risks associated with spread of the organism to surfaces, hands and other foods. This has required the development of packaging that is sufficiently robust to withstand oven cooking and whilst it is a useful addition to reduce risk, this type of chicken remains relatively low volume and many customers are in the habit of opening the packs (personal communication).

Labelling

5.112 A significant potential risk associated with chicken is undercooking and cross contamination. For many years food safety advice has been provided on retail packaging regarding the cooking times and temperatures required to cook a product

safely and also additional advice on visual inspection to verify that the product has been suitably cooked e.g. piping hot and the juices run clear when pierced with a fork. In addition, many retailers provide advice on storing and preparing raw poultry in a way that prevents cross contamination i.e. on separate surfaces, with separate utensils for raw and ready to eat foods. A very common historic consumer practice in the UK has been to wash chicken as part of the preparation of the bird prior to cooking. The high numbers of *Campylobacter* spp. on the surfaces of a chicken would indicate that the washing of a bird in water and the creation of a large volume of aerosols is likely to spread contamination onto surfaces, hands and potentially other foods. Advice has therefore been added to retail packaging to avoid washing raw chickens, due to the increased risk of cross contamination and this has been further promoted by the Food Standard Agency.

Consumer acceptability

5.113 Interventions applied for the reduction of *Campylobacter* spp. on chicken must be acceptable to the consumer both in terms of real and perceived risk. A number of interventions such as irradiation and chemical washes have rarely found acceptance even where consumers have an understanding of the microbiological food safety risk (557).

5.114 *Campylobacter* spp. on chicken will continue to present a burden to the consumer despite significant improvements in farming and industry controls and therefore post production mitigation factors ('leak-proof' packaging, modified atmospheres, consumer advice) need to continue to be investigated to reduce the risk to the consumer.

Collaboration

5.115 A key feature in the reduction of *Campylobacter* spp. achieved in retail poultry in the UK has been the full voluntary supply chain approach taken by farmers, processors and retailers supported by government and enforcement bodies and the academic community. The development of targets for the reduction in contamination, the sharing of research and development on intervention studies and the impact of full-scale mitigations e.g. farm controls, thermal processing, etc. on

achieving the target underpinned the reductions achieved by the industry. This collaborative approach was undertaken initially under the auspices of a Joint Working Group that was subsequently replaced by the 'Acting on *Campylobacter* Together' (ACT) Board. The approach taken by regulators such as the Food Standards Agency in facilitating cross functional working was welcomed by the industry but some aspects were considered to compromise effective collaboration such as the publishing of 'name and shame' style league tables and the promotion of a competitive element which progressively undermined open sharing. This is an important lesson with regard to future ways of working on topics that need a full chain solution.

5.116 A key factor in the initial success achieved by the industry in reducing the levels of *Campylobacter* spp. in UK chicken was a full supply chain approach and the importance of promoting an open, collaborative approach is recommended for this and other industry challenges.

Conclusion and recommendations

5.117 No single practical intervention has been shown to be capable of eliminating *Campylobacter* spp. or even reducing it to acceptable levels in the bird or during processing. Evidence, however, does show that levels can be reduced by a combination of farm and processing controls that include implementation of improved biosecurity measures on farm e.g. hygiene barriers in sheds, time-controlled depopulation and in the process e.g. optimisation of existing processing, application of thermal processing (hot or cold). This has been shown to be capable of reducing contamination significantly in recent years from a position where over 30% of chickens on retail sale in the UK had >1000$\log_{10}$ cfu per g of *Campylobacter* spp. on the skin to <7%. This has required major investment in resource and capital but further progress will need to be made to ensure the burden presented by chicken to consumers reduces further.

Farming

5.118 *It is recommended that the farming industry continues to implement high measures of biosecurity incorporating all of the elements to reduce opportunities for the introduction of* Campylobacter *spp. into the broiler house. In addition to the*

recognised controls of litter, water, feed, animals, human activity, flies, etc. the following areas are potentially of significant additional benefit and should receive further consideration for adoption/evaluation by the industry: litter moisture control; time managed thinning; hygiene barriers; antimicrobial factors (microbiome and bacteriophage).

Processing

5.119 *Several processing techniques have been demonstrated to achieve significant reductions in* Campylobacter *spp. One of the most important elements in reducing contamination in the processing plant is optimisation of current processing equipment to minimise spread of contamination e.g. plucking and to reduce contamination e.g. inside outside washing. Other technologies that have shown promise and where the industry is recommended to continue adoption and further investigation to enhance efficacy include thermal processing (water and steam) and rapid surface chilling.* ***It is recommended that the factors leading to reduction in* Campylobacter *spp. during the shelf life of the product should be elucidated as this may provide opportunities for additional controls.***

Consumers

5.120 *The continued presence of* Campylobacter *spp. on chicken necessitates the ongoing education of the consumer in cooking and cross contamination controls.* ***It is recommended that the FSA continues to highlight these controls to consumers and industry provides clear labelling advice on storing, preparing, handling and cooking of chicken.***

Collaboration

5.121 ***A key factor in the initial success achieved by the industry in reducing the levels of* Campylobacter *spp. in UK chicken was a full supply chain approach and the importance of promoting an open, collaborative approach is recommended for this and other industry challenges***.

5.122 ***Much of the improvement in farm and processing measures to reduce the colonisation and contamination with* Campylobacter *spp. has been undertaken in the large poultry processing sector and it is recommended that***

the FSA, industry assurance and sector bodies ensure that all farms and processors involved in the production of chicken are encouraged to adopt similar standards.

Chapter 6: Risks in the Food Chain: Measures to prevent *Campylobacter* contamination of chicken meat in Europe, New Zealand and the USA

Introduction

6.1 Animal and human health surveillance data, together with research reports, suggest that the incidence of *Campylobacter* in commercially-reared chickens in some EU Member States (MS), particularly Scandinavian countries, is lower than in the UK and the systems employed by them may inform UK interventions.

6.2 The following section gives an overview of *Campylobacter* infection rates in each of the countries along with details of *Campylobacter* interventions employed by the Competent Authority in each territory in the reduction of *Campylobacter* levels, for comparison information is also given for the UK, New Zealand and the USA.

European Commission

6.3 *Campylobacter* is acknowledged as a concern in broiler production across Europe and the European Commission has initiated preliminary talks with Member States on a number of measures related to the control of *Campylobacter*. The main measure is the introduction of a process hygiene criterion (PHC) for *Campylobacter* in broilers (EU 2017/1495), but the UK is far ahead of many MS in reducing *Campylobacter* levels in poultry meat. Whilst many MS accept the need for PHC to help reduce levels of *Campylobacter,* they are less ambitious than the UK regarding the criteria, and current targets of <40% >1000 reducing to 20% by 2025 are well above those set voluntarily by food business operators as part of the FSA campaign, 'Acting on *Campylobacter* Together'.

Commented [MU4]: Amended to reflect Moy Park's consultation comments

Commented [MU5]: Amended to reflect Moy Park's consultation comments.

6.4 New EU official control regulations are being negotiated at which time the Commission will be required to review and amend Regulation 854/2005 which includes specific controls on the production of fresh meat. At the same time, the Commission is also discussing the authorisation of peroxyacetic acid (PAA) as a poultry wash to reduce the presence of surface contaminants, including

Campylobacter. Whilst central action by the Commission is still on-going, a number of European Nations have undertaken their own interventions and these are detailed below.

UK

Human *Campylobacter* infection in the UK

6.5 There was an increase in the number of human *Campylobacter* cases in the UK in 2014, in comparison with 2013, the annual incidence being around 104 cases per 100,000 of the population. Despite considerable work by industry in partnership with the UK government and others through various initiatives including ACT (Acting on *Campylobacter* Together) cases of *Campylobacter* in the UK remain stable (96.9 per 100,000 in 2015, 89.8 per 100,000 in 2016 and 96.8 per 100,000 in 2017)(558).

Characteristics of UK broiler industry

6.6 The UK broiler industry processes around 950 million birds per year, giving a total production of around 1460000 tons of chicken meat.

6.7 The industry comprises 4 main producers, who make up around 80% of the UK chicken production, running an estimated 1,500 to 2,000 farms.

6.8 The UK operates thinning and has opted for a maximum stocking density of 39kg/m^2.

Interventions

6.9 Whilst there is no set intervention in the UK, producers have been working with retailers, government and academia to find ways of reducing contamination throughout the food chain.

6.10 In August 2009, the Joint Working Group on *Campylobacter* was established as a joint industry and government group. It aimed to identify interventions that would reduce *Campylobacter* in chicken. Membership included all the major retailers and producers along with the British Poultry Council (BPC), the National Farmers' Union (NFU) the British Retail Consortium (BRC), the FSA and Defra.

6.11 This group was superseded in 2014 by the ACT Board. The Food Standards Agency, Defra, the UK poultry industry, and major retailers agreed a new target to measure efforts to reduce the levels of *Campylobacter* in chickens.

6.12 There are three categories of contamination level (low = under 100cfu/g, medium 100-1000cfu/g and High = over 1000cfu/g) The target was for the industry to reduce the numbers of these most contaminated birds in UK poultry houses from 27% to 10% by 2015. It was estimated that achievement of this target could mean a reduction in *Campylobacter* food poisoning of up to 30% – about 111,000 cases per year.

6.13 Acting on Campylobacter Together is a campaign to bring together the whole food chain to reduce levels of *Campylobacter* in chicken and to reduce the burden of foodborne illness in the UK.

6.14 The Acting on *Campylobacter* Together accelerated solutions event was held in June 2014. It brought together representatives from government, retailers, caterers, poultry producers and processors, and consumer organisations, to agree actions that could be taken to reduce *Campylobacter*. As part of the event, a pledge was developed which allowed organisations to demonstrate their commitment to the campaign.

The pledge

The human impact of campylobacter is unacceptable.

Tackling campylobacter is a critical priority for our organisation.

We commit to acting now to ensure we achieve the 2015 target and to delivering a future in which campylobacter in poultry is no longer a threat to human health.

As part of this commitment we will:

- *share legally all information we have that could help make a difference*
- *invest as much time, effort and money as it takes*

6.15 In addition, a separate consumer organisation pledge was signed to reflect the commitment of consumer organisations to the campaign.

Consumer organisations' pledge

The human impact of campylobacter is unacceptable.

We are committed to doing all that is in our scope to encourage tougher action to bring down levels in chickens and ultimately reduce the high rates of unnecessary food poisoning it causes.

Further information on the pledges can be found on the FSA website:
https://www.food.gov.uk/news-updates/campaigns/campylobacter/actnow#toc-4

6.16 Whilst Industry did not manage to meet the target of the highest level of contamination of birds being below 10% by the end of 2015, renewed efforts and the introduction of various interventions at processing have led to greater reductions in the level of contamination and the latest figures from the UK survey of whole UK chickens shows that on average, across the market, 6.5% of chickens tested positive for the highest level of contamination.
https://www.food.gov.uk/news-updates/news/2017/16235/further-reduction-levels-of-campylobacter-chicken

Europe

Denmark

Human *Campylobacter* infection in Denmark

6.17 There has been a small increase in the number of human *Campylobacter* cases in Denmark in 2014, in comparison with 2013, the annual incidence being around 67 cases per 100,000 of the population. There is an approximate 50:50 split between the number of cases acquired in Denmark and those acquired abroad.

Source - The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2014
http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2015.4329/full

6.18 The most significant sources of infection are:

- poultry meat;
- pork and beef;
- polluted drinking water; and
- contact with domestic cats and dogs.

6.19 There is a much more pronounced summer peak in human infection than in the UK. The consumption of poultry meat is a significant risk factor and the Danes have carried out a risk assessment which shows that, where the number of *Campylobacter* on chicken carcasses is reduced by freezing or other means, the risk of human infection is also reduced.

Characteristics of the Danish broiler industry

6.20 The production of about 126 million broilers annually takes place in an industry based on private ownerships within the individual links of the production chain. The entire broiler production in Denmark is based around 1 hatchery (2 locations but same owner) that distributes to 180 broiler producers. Birds are then slaughtered at either small independent slaughterhouses or supplied to two major broiler processing plants – Rose Poultry A/S and Lantmännen Danpo A/S.

6.21 106 million are slaughtered within the Danish system, with the remaining 10 million are exported alive for slaughter outside Denmark.

6.22 Denmark has very hot summers and this presents particular difficulties for on-farm control. It is not uncommon for some broiler houses to be left open in summer for welfare reasons, and this undermines biosecurity. The current aim is therefore to reduce flock colonisation rather than to eliminate it.

6.23 Less than 20% of total broiler production is thinned and Denmark operate a maximum stocking density of 42kg/m^2.

Source - Danish Poultry Meat Association - https://www.foedevarestyrelsen.dk/SiteCollectionDocuments/25_PDF_word_filer%20til%20download/05kontor/Servicetjek_Fjerkraesektoren/7_%20Introduction-to-the-dansih-broiler-sector-Birthe-Steenberg-Landbrug-og-Foedevarer.pdf

Interventions

6.24 All poultry flocks in Denmark are subject to surveillance to determine their *Campylobacter* status. The Danish monitoring programme stipulates that all flocks must be tested at the processing plant, 24 caeca samples are tested per flock. *Campylobacter* positive flocks are frozen or heat treated before consumption. Biosecurity is felt to be a very important intervention and processors penalise farmers who supply them with *Campylobacter*-positive birds, by reducing the live weight price paid to the farmer. This is used as a way of offsetting the cost to the processor of treating the contaminated carcass and forces the farmer to operate to the strictest levels of biosecurity.

Sweden

Human *Campylobacter* infection in Sweden

6.25 There has been an increase in the number of human *Campylobacter* cases in Sweden in 2014, in comparison with 2013, the annual incidence being around 86 cases per 100,000 of the population. There is an approximate 50:50 split between the number of cases acquired in Sweden and those acquired abroad.

6.26 With the exception of some large waterborne outbreaks, chicken meat is regarded as the most common source of *Campylobacter* infection acquired in Sweden. The most common risk factors identified in outbreaks are:

• eating chicken meat
• poultry contact at work or at home;
• contact with lake/stream water;
• domestic well water; and
• raw drinking milk.

Source - The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2014
http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2015.4329/full

Characteristics of the Swedish broiler industry

6.27 Broiler grandparents (Ross and Cobb) are imported from the UK. Approximately 83 million broilers are reared annually, and they are slaughtered at the approximate age of 35 days. Broilers are reared on litter and "all-in-all-out" management is practiced at both flock- and farm level.

6.28 A differentiated population density allows a maximum bird density at the end of the growing period of 36 kg or 25 birds per m^2. The majority of Swedish flocks are not thinned.

Source - The National Veterinary Institute, SVA. http://www.sva.se/en/animal-health/poultry

6.29 The Swedish broiler industry comprises 7 companies with 8 slaughterhouses and a total of 124 farms with approximately 500 broiler houses in total. Average flock size is around 30,000 birds (maximum 50,000). The newer farms generally have flocks of 50,000 birds and 2-4 houses or compartments. Broiler houses are classified for welfare purposes in Sweden and stocking density is a key feature of the classification arrangements. Only the best houses are permitted to be stocked to the maximum density (36kg/m2). Most houses are stocked to a density of at least 33 kg/m2. Stocking density in houses with low welfare scores is restricted to 20kg/m^2.

Interventions

6.30 Sweden is a very important element in any consideration of Scandinavian broiler production. Biosecurity features include the requirement to change clothing

and footwear at the entrance to each house, and an all-in-all-out production system across the entire farm.

6.31 The Swedish *Campylobacter* Monitoring Programme stipulates that all testing is carried out at the processing plant. Ten caeca samples are tested per house and positive flocks must be heat treated or frozen before consumption. The *Campylobacter* monitoring programme has been very successful in Sweden with flock prevalence dropping from 60% in 1989 to 8.8% in 2013. Like Norway and Denmark, processors penalise farmers who supply them with *Campylobacter*-positive birds, by reducing the live weight price paid to the farmer as a way of offsetting the cost to the processor of treating the contaminated carcass. This has the secondary benefit of encouraging farmers to operate to the strictest level of biosecurity.

The Netherlands

Human *Campylobacter* infection in the Netherlands

6.32 There has been a marked increase in the number of human *Campylobacter* cases in the Netherlands in 2014, in comparison with 2013, the annual incidence being around 48 cases per 100,000 of the population.

Source - The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2014 http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2015.4329/full

Characteristics of Dutch broiler industry

6.33 For poultry meat production 44.1 million broiler chickens are kept at 700 farms, Gross domestic production of broilers is around 625.000 tonnes (carcass weight).

Source - World Poultry Science Association - http://wpsa-foodsafety.com/index.php?item=161

Interventions

6.34 In The Netherlands, the industry has adopted its own private Process Hygiene Criterion (started in April 2014). This is a private criterion and authorities do not have 'legal access' to control if the slaughterhouses fail to fulfil the criteria; however, they are willing to share results with the Association of Dutch Poultry Processing Industries NEPLUVI and to discuss corrective actions should the criteria be missed. Under this private scheme 5 breast skin and 3 caeca are taken per batch and the prevalence is given as % breast skin at levels above 1000 cfu/g and % breast skin at 10,000 cfu/g along with % positive flocks (derived from caeca samples).

6.35 NEPLUVI is participating in the EU funded CAMPYBRO project which is investigating if there are any additives that can be added to poultry feed to reduce *Campylobacter* in live birds (further details below).

6.36 NEPLUVI is also an investigator in the Dutch 'PPS-Campylobacter de Baas' project along with University of Utrecht, Wageningen University and poultry sector stakeholders on the control of *Campylobacter* in poultry production (started in 2015, reports 2018).

Source – Private email from NEPLUVI
Source - http://www.wageningenur.nl/en/project/Campylobacter-de-baas.htm

CAMPYBRO project

6.37 The **CAMPYBRO** project, funded under the 7th FRAME PROGRAM of EU (Capacities- Research for SME Associations FP7-SME GA 605385) has the objective of developing practical strategies to decrease the population of *C. jejuni* in broilers through two strategies - nutrition and vaccination. The project started in September 2013 and finished in August 2016. The project is coordinated by IMASDE AGROALIMENTARIA S.L. (IMASDE), and has 10 partners: 5 poultry producers National Associations (**FIA** and **CIDEF** from France, **PROPOLLO** from Spain, **NEPLUVI** from The Netherlands, **BTT** from Hungary), a vaccine laboratory (CZV, Spain), a poultry producer (**EXPLOTACIONES AVÍCOLAS JOSÉ LUIS REDONDO S.A.**, Spain) and a laboratory (**MIKROLAB**, Hungary). The Research centers involved are **ANSES** (France) and **IMASDE** (Spain).

Iceland

Human Campylobacter infection in Iceland

6.38 There was a marked increase in the number of human *Campylobacter* cases in Iceland in 2014, in comparison with 2013, the annual incidence being around 44 cases per 100,000 of the population. There is an approximate 50:50 split between the number of cases acquired in Iceland and those acquired abroad.

Source - The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2014
http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2015.4329/full

Characteristics of Icelandic broiler industry

6.39 The Icelandic broiler industry processes around 4.8 million birds per year, giving a total production of around 7,200 to 7,600 tons of chicken meat. Iceland does not export any chicken; all production is for home consumption.

6.40 The industry comprises 3 main producers running integrated production (parent stock, hatchery, rearing, slaughter and processing) i.e.: 3 slaughterhouses and 3 cutting plants. Chicken is reared on 27 broiler farms, with total of 83 houses. Total capacity of 730,000 broilers per run.

6.41 Iceland operates a no-thin policy and has opted for a maximum stocking density of 39kg/m^2.

Source - Presentation by Icelandic Food and Veterinary Authority -
https://www.norden.org/no/nordisk-ministerraad/ministerraad/nordisk-ministerraad-for-fiskeri-havbruk-jordbruk-naeringsmidler-og-skogbruk-mr-fjls/institusjoner-samarbeidsorganer-arbeidsgrupper-og-prosjekter/nordisk-arbeidsgruppe-for-mikrobiologi-dyrehelse-og-dyrevelferd-nmdd/arrangementer/dyrevelfaerd-for-kyllinger-der-holdes-med-henblik-paa-koedproduktion/brigitte-brugger-chicken-welfare-in-iceland-nmdd-stockholm

Interventions

6.42 The Icelandic Monitoring programme, which was instigated in 2000, stipulates that all poultry products must be cooked or frozen unless it can be proven that the bird was free from *Campylobacter*. Ten faecal samples are collected no more than 5 days prior to slaughter and tested, if there is no result or samples go missing all meat

within batch is frozen, as is any meat from flocks that test positive, all such meat is frozen for a minimum of 3 weeks. Between April and October producers are also required to test at processing to monitor the number of flocks that turn positive between testing on farm and processing, if birds test positive at any stage distribution is stopped and the meat is frozen.

6.43 Biosecurity on farm is important and Industry explored paying financial incentives to farmers whose flocks test negative and penalties to flocks that test positive, but this has now been stopped as it had no noticeable benefits.

Norway

Human *Campylobacter* infection in Norway

6.44 There has been an increase in the number of human *Campylobacter* cases in Norway in 2014, in comparison with 2013, the annual incidence being around 66 cases per 100,000 of the population. There is an approximate 50:50 split between the number of cases acquired in Norway and those acquired abroad.

Source - The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2014 - http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2015.4329/epdf

6.45 There is a very marked peak in the incidence of human infection, with approximately 75% of all cases occurring in July, August, and September. It is thought that many more cases are caused by contaminated water in Norway than in the UK. The consumption of poultry purchased raw is among the other principal risk factors. Principal vehicles of infection are:

- the consumption of non-disinfected water;
- the consumption of poultry purchased raw;
- attending outdoor barbeques; and
- professional contact with animals.

Characteristics of Norwegian broiler industry

6.46 The industry is approximately 10% of the size of that in the UK and, in general, birds are killed at 32-35 days of age. Norway has a national programme for the

surveillance of *Campylobacter* in poultry flocks, which is funded by the Government and the industry.

6.47 In Norway, broilers are raised in one of around 550 farms each normally operating one house, the average flock size is around 17,500 birds (2016 data). Norway has a minimal free range/organic industry. Birds are processed at one of 5 slaughterhouses.

6.48 The majority of broiler flocks are not thinned in Norway and the standard stocking density is 36kg/m^2.

Source – National Veterinary Institute Presentation 2006 - http://www.sva.se/globalassets/redesign2011/pdf/om_sva/nrl/crl/presentationer/bjarne_bergsjo_presentation.pdf

Interventions

6.49 Given the rising incidence of human campylobacteriosis, and the association with the consumption of poultry meat, Norway introduced an Action Plan Against *Campylobacter* in Broilers. The plan was developed by the Norwegian Zoonosis Centre and had three goals:

- reduce the human exposure to *Campylobacter* from Norwegian poultry;
- improve food safety; and to
- reduce the incidence of human campylobacteriosis associated with Norwegian poultry.

6.50 The Norwegian Monitoring Programme stipulates that between May and October all flocks should be tested. Ten Faecal samples are taken from all broiler flocks within a maximum of 5 days of processing and tested by PCR. All carcases from *Campylobacter* positive flocks must be heat treated or frozen for a minimum of 3 weeks. The farmers will also receive a consultation/ advisory visit.

6.51 The programme also stipulates that it is mandatory for all well and borehole water used for bird drinking to be UV-treated before it is given to broilers and all drinker lines must be sanitised to remove biofilms. Industry has also adopted the

good practice of processing positive flocks through the slaughter line at the end of the day (where possible).

6.52 Biosecurity on farm is seen as key and processors penalise farmers who supply them with *Campylobacter*-positive birds, by reducing the live weight price paid as a way of offsetting the cost to the processor of treating the contaminated carcass. This penalty also pushes the farmer to operate to the strictest level of biosecurity.

Rest of the world

New Zealand

Human *Campylobacter* infection in the New Zealand

6.53 Unlike the other countries mentioned above, New Zealand, not being part of the EU or EEA, do not submit returns to the European Union summary report on Trends and Sources of zoonoses, zoonotic agents and foodborne outbreaks. However, some data are available through the New Zealand Medical Journal and the ESR Surveillance report - Notifiable and other diseases in New Zealand.

6.54 Campylobacteriosis is the most common notified disease in New Zealand with 6213 cases in 2015 comprising 43.5% of all notifiable diseases reported to Public Health Services.

6.55 Campylobacteriosis in New Zealand peaked at 396 reported cases per 100,000 population in 2003; the highest rate reported by any developed country in the world. The incidence remained at this level until 2006 when it dropped rapidly over a 2-year period to 157 reported cases per 100,000 population by 2008, a considerable decrease on the preceding decade.

6.56 The 2015 rate of 135.3 per 100,000 was significantly lower than the 2014 rate of 150.4 per 100,000 (6782 cases). Consumption of food from retail (food) premises and contact with farm animals were the most common risk factors for campylobacteriosis. In 2015, 19 outbreaks of campylobacteriosis were reported involving 88 cases.

Source - https://surv.esr.cri.nz/PDF_surveillance/AnnualRpt/AnnualSurv/2015/2015AnnualReportFinal.pdf

Characteristics of the New Zealand broiler industry

6.57 Poultry meat production in New Zealand for the year 2014 was around 190,000 tonnes. The legal maximum stocking density is 38 kg per m^2.

Source - Poultry Industry Association of New Zealand. - http://pianz.org.nz/industry-information/industry-statistics

6.58 In 2008 it was recorded that 158 farms produced chickens.

Source - http://www.foodsafety.govt.nz/elibrary/industry/farm-factors-campylobacter-research-projects/FW0767_On_farm_factors_survey_report_May_2008_web.pdf

Interventions

6.59 In New Zealand the *Campylobacter* Performance Target (CPT) was introduced by NZFSA (now the Ministry for Primary Industries (MPI)) to put the responsibility on the poultry industry to implement and achieve set targets. This came about following reports implicating poultry as the biggest source of food borne campylobacteriosis in NZ. Since 2007 there has been a mandatory testing system in all primary broiler processing plants in NZ, with the full implementation of the CPT from 2008. The pressure is primarily on processing to reduce the levels of *Campylobacter*. Results over the last few years have confirmed that improvement in hygienic processing has resulted in sizeable reductions of *Campylobacter* on poultry.

6.60 All test results are added to the MPI's National Microbial Database, which provides data needed to assess the effectiveness of interventions and analyse trends. MPI and the industry have a *Campylobacter* response team who will visit a plant where ongoing non-compliance occurs. Should the processor fail to reduce the level of *Campylobacter* the MPI response team will get involved and this could lead to a number of corrective actions as appropriate, and in the worst case, premises closure.

Testing in processing plants

6.61 Mandatory testing in processing plants was introduced in 2007 and is centred on an outcome-based result. The MPI set the standard the industry needs to achieve and it is up to the industry to make sure that they implement and manage the interventions to achieve the CPT targets. It was decided that the whole-carcass rinse would be used as the standard testing method because the United States Department of Agriculture used this method and it thus allowed NZ to compare its results with those of the USA.

6.62 The whole-carcass rinse test involves placing a random carcass in a bag with 400 ml of solution and shaking the bag for 2 minutes while massaging the surface. The fluid is then poured into a sealed container and sent to a laboratory for testing. The level of *Campylobacter* in each sample is determined. A *Campylobacter* count of greater than 3.78 log10 is counted as positive. In addition, there is a detection target. Having more than 29 of the 45 samples with detectable *Campylobacter* is also counted as positive. The limit of detection is 2.3 $\log_{10}$ per carcass.

6.63 The number of samples taken by each plant depends on the number of chickens a plant processes each year. Plants processing fewer than 1 million birds per year are required to take 3 random samples each week. Plants processing more than 1 million birds per year have to take 3 samples per day. With 3 samples each day over 5 processing days there will be 15 samples taken per week. There is then a 3-week moving window in which the total number of positive samples out of the 45 taken is monitored. The idea behind the moving window is to give companies the opportunity to rectify problem areas within the 3 weeks. Should any plant exceed either the enumeration limit of 7 out of 45, or the detection limit of more than 29 out of 45 samples in any 3-week period, then it would be classed as a non-compliance and triggers an internal investigation. The company will look at its processes to identify any shortcomings. Should there be subsequent weeks of non-compliance, an escalating response will take place.

Source - http://www.foodsafety.govt.nz/industry/general/nmd/

USA

Human *Campylobacter* infection in the United States

6.64 As with New Zealand, the USA are not required to submit returns to the European Union summary report on Trends and Sources of zoonoses, zoonotic agents and foodborne outbreaks. However, some data are available through the CDC – Centre for Disease Control.

6.65 *Campylobacter* is one of the most common causes of diarrheal illness in the United States. Most cases occur as isolated, sporadic events, not as part of recognized outbreaks. Active surveillance through the Foodborne Diseases Active Surveillance Network (FoodNet) indicates that about 14 cases are diagnosed each year for each 100,000 persons in the population. Many more cases go undiagnosed or unreported, and campylobacteriosis is estimated to affect over 1.3 million US citizens every year

Source – CDC - http://www.cdc.gov/foodsafety/diseases/campylobacter/index.html

Characteristics of the US broiler industry

6.66 The United States has the largest broiler chicken industry in the world, and about 19% of production was exported to other countries in 2015 (top importers Mexico, Canada and Hong Kong).

6.67 Poultry meat production is at around 9 billion broiler chickens, these are reared on over 25,000 farms that supply the 186 processing plants (owned by 35 major companies) in the US.

6.68 Gross domestic production of broilers in the US for the year 2015 was estimated at 53 billion pounds, liveweight. More than 40 billion pounds of chicken product was marketed, measured on a ready-to-cook basis.

Source - http://www.nationalchickencouncil.org/about-the-industry/statistics/broiler-chicken-industry-key-facts/

6.69 The Council for Agricultural Science and Technology (CAST) states that the minimum space requirement is one-half square foot per bird.
Source – CAST - http://www.cast-science.org

Interventions

6.70 The Food Safety and Inspection Service (FSIS), is the USDA department that implements and monitors the *Campylobacter* Performance Standards in the USA. According to the FSIS website the *Salmonella* and *Campylobacter* Performance Standards apply to processing plants' overall process control, not to individual products. Products are not tested to measure their disposition, but rather to measure the effectiveness of the slaughter and grinding process in limiting contamination. *Campylobacter* Performance Standards are taken in sets and the results of an entire set are used to determine if a processing plant is meeting the Performance Standard (FSIS, 2014). The current Performance Standards have been in place since January 2011.

Testing in processing plants

6.71 Sampling is carried out by FSIS staff in all US processing plants and samples are collected after the carcasses exit the immersion chillers. The set is started unannounced so that processing plants have to ensure their interventions are working and effective every day of the year.

6.72 When a set has started, a random carcass is selected and tested for the presence of *Campylobacter*. This process is repeated daily for 51 consecutive days. Carcasses are collected and samples are obtained by placing a random carcass in a bag and rinsing the carcass with 400ml of solution. 100ml of the fluid is poured into a sealed container and sent to the laboratory for testing. Testing is carried out to determine if any *Campylobacter* is present in the sample. Any *Campylobacter* found in the sample counts as positive. To achieve the Performance Standard for whole carcasses, a plant must have fewer than 8 positive samples out of 51. If a plant has 8 or more positive samples, they fail the Performance Standard.

Categorisation of plants

6.73 Depending on the number of samples that test positive in the two most recent sets, the processing plant falls into one of 3 categories.

- For whole carcasses, a processing plant is in Category 1 if no more than 4 out of 51 samples test positive i.e. less than 50% of the Performance Standard in both sets.

- A processing plant is in Category 2 if between 5 and 7 samples test positive i.e. between 50% and the Performance Standard in both sets.

6.74 If a plant fails to meet Category 1 standard for only one of the two sets the plant falls into Category 2. This allows the plant to return to Category 1 standard should the next set meet the criteria.

- A processing plant is in Category 3 if more than 8 samples test positive i.e. more than the Performance Standard in one out of the two most recent sets.

6.75 The aim is for all a company's processing plants to fall into Category 1. Companies must inform their customers should they move out of Category 1, which could lead to orders being cancelled from that plant. Occasionally there can be several months between sets so if a plant has slipped back to Category 2 it could cause the company a problem for a long time.

6.76 Recently, FSIS posted the progress report for the period May 3, 2015–June 25, 2016 which stated that 90.1% of broiler processing plants were in Category 1, 1.1% in 2T, 3.3% in 2, and 5.5% in Category 3.

Source - http://www.fsis.usda.gov/wps/portal/fsis/topics/data-collection-and-reports/microbiology/salmonella-verification-testing-program/establishment-categories

Changes to the Performance Standards

6.77 The USDA Performance Standards changed in March 2015. Instead of taking a sample on 51 consecutive days a random sample is taken every day throughout the year. The results are assessed on a rolling window so that the results will always be measured against the last 51 samples. There are different targets to meet depending on the type of poultry meat that is produced.
In the past, the main focus was on reducing the number of pathogens on whole carcasses; however, this has changed to include portions and ground poultry meat (FSIS, 2015). Four pounds (lbs) of parts and 325 grams of comminuted product are collected and tested for *Campylobacter* and *Salmonella*. Since 4 lbs of chicken wings will come from more than 20 different birds, the Performance Standards are harder to meet.

6.78 As with whole carcasses, the number of positive samples of portions and ground chicken that test positive in the set puts the processing plant in to one of the 3 categories.

Source – USDA Website -
http://www.fsis.usda.gov/wps/portal/fsis/home/!ut/p/a0/04_Sj9CPykssy0xPLMnMz0vMAfGjzOINAg3MDC2dDbwsfDxdDDz9AtyMgnyMDf3dDPQLsh0VAcy6FX0!/?1dmy&page=gov.usda.fsis.internet.newsroom&urile=wcm%3Apath%3A/fsis-content/internet/main/topics/data-collection-and-reports/microbiology/salmonella-verification-testing-program/salmonella-verification-testing-program

Table 6.1 Measures to prevent *Campylobacter* contamination of chicken meat in Europe, New Zealand and the USA

Country	Human cases per 100,000	Birds processed per year	Tons of meat produced	Number of Processors	Number of farms	Stocking density	Intervention – number of samples	Intervention – positive samples.
UK	104 (2014)	953.1 million (2015)	1456400 pa (2015)	4 Major processors (80% Market)			N/A	N/A
Iceland	44 (2014)	4.8 million	7200 – 7600 pa	6 (3 slaughter-houses and 3 cutting plants)	27	39kg/m2	10 faecal samples per flock	Freeze for minimum 3 weeks
Denmark	67 (2013)	126 million			180	42kg/m2	24 caeca samples per flock	Freeze or heat treatment
Norway	66.3 (2014)	73.9 million (2014)	93.5 (2014)	5 Slaughterhouses	699 (2014) 550 (2016)	36 kg/m2	10 faecal samples per flock	Heat treatment or frozen min 3 weeks
Sweden	86 (2014)	83 million		7 companies (8 slaughterhouses)	124	36kg/m2	10 caeca samples per house	Heat treatment/ freezing
Netherlands	48 (2014)	44.1 million	625000 pa		700		5 breast & 3 caeca per batch	Further treatment
New Zealand	159 (2012)		190000 pa		158	38kg/m^2	1 Whole carcass rinse	Intervention, if positives continue, farm closure
USA	14 (2012)	9 Billion pa	53 billion lbs	186 processing plants	25,000			

Chapter 7: Red meat, raw milk and fresh produce

7.1 Red meat (pork, beef, lamb and other red meats)

Market statistics (volume, value, imports)

7.1.1 Overall, red meat accounted for 58% of UK meat consumption in 2015 (559, 560). Although poultry was the single most eaten meat in the UK in 2015 (refer to Chapter 6), both in terms of overall consumption (Table 7.1) and *per capita* consumption (Table 7.2), this accounted for only 42% of total meat consumption in the UK. Red meat consumption in 2015 comprised pork (30%), beef (22%) and lamb (6%). Analysis of *per capita* consumption data for the period 2012 to 2015 (Table 7.2) indicates that total meat consumption increased by 5% with this trend largely accounted for by an increase in poultry consumption (7.6%). Lamb consumption increased markedly (16.2%) but retained only a small market share, while pork and beef consumption increased only slightly (1.6% and 1.1% respectively).

Table 7.1 Meat consumption in the UK, 2012 – 2015 *(559, 560)*

(000 tonnes cwe)	Beef and veal	Lamb and mutton	Pig meat	Poultry	Total meat
2012	1,144	277	1,564	2,091	5,075
2013	1,108	296	1,532	2,103	5,039
2014	1,149	298	1,578	2,130	5,154
2015	1,182	327	1,629	2,308	5,446

Note: Total supplies of meat available for consumption, both in and outside the home. Includes imported meat products.

Source: Defra

Table 7.2 *Per capita* consumption, UK, 2012-2015. *(559, 560)*

(kg cwe)	Beef and veal	Lamb and mutton	Pig meat	Poultry	Total meat
2012	18.0	4.3	24.6	32.8	79.7
2013	17.3	4.6	23.9	32.8	78.6
2014	17.8	4.6	24.4	33.0	79.8
2015	18.2	5.0	25.0	35.5	83.7

Note: Includes meat in meals eaten both in and outside the home. Includes imported meat products.

7.1.2 Data on UK self-sufficiency rates (Table 7.3) indicates that the UK production met approximately three quarters of the demand for poultry in 2015 (declining from 76.9% in 2012 to 73.3% in 2015). Self-sufficiency in beef production closely matched poultry (declining from 77.4% in 2012 to 74.7% in 2015). In contrast, in recent years,

the UK produced only slightly over half of its entire demand for pig meat (increasing from 52.7% in 2012 to 55.1% in 2015). Finally, the UK has historically been self-sufficient in lamb production but a decline was noted from 99.5% in 2012 to 91.9% in 2015.

Table 7.3 Self-sufficiency rates, UK, 2012 – 2015. *(559, 560)*

%	Beef and veal	Lamb and mutton	Pig meat	Poultry	Total meat
2012	77.4	99.5	52.7	76.9	70.8
2013	76.4	97.8	54.4	79.0	72.0
2014	76.4	99.9	54.7	77.3	71.5
2015	74.7	91.9	55.1	73.3	69.3

Contamination sources in pork, lamb and beef production

7.1.3 Contamination of red meat and offal, including liver, by *Campylobacter* sp. arises from direct or indirect contact with faeces or intestinal contents during slaughter and processing. However, it is important to note that other pathways exist, beyond consumption of meat, for transmission of *Campylobacter* from red meat livestock species including faecal contact through direct occupational exposure, environmental transmission and milk-associated and other food pathways.

7.1.4 The arrival at the abattoir of livestock already carrying intestinal *Campylobacter* lies at the root of subsequent contamination of meat. Most, if not all, pigs are colonised by thermophilic *Campylobacter* at some stage in their lives with *C. coli* being more frequently detected than *C. jejuni* amongst an array of other *Campylobacter* species (561). Prevalence of carriage of *Campylobacter* species in surveys of faeces or intestinal content of slaughter pigs ranged from 90% in the USA (562) and Denmark (563), through 69% in the UK (564) down to 36% in Japan (565) and 26% in Ireland (566). The opportunity for *Campylobacter* shedding and contamination occurs at several points along the standard processing chain. Pigs are transported from production farms to processing plants where they may be lairaged for up to 48 hours prior to slaughter. Pig processing involves electrical or carbon dioxide stunning followed by killing through exsanguination. Carcases are then subjected to a scalding, dehairing and polishing process prior to evisceration which follows a two-step process whereby the carcass is suspended head down while the abdominal viscera are removed followed by the thoracic viscera. Carcasses then undergo chilling at <7^{o}C with many plants applying a preliminary blast chill to support surface drying and

temporary achievement of sub-zero temperatures at the carcass surface. One US study of pigs moving from farms though slaughter and processing found a high prevalence and shedding load in faeces while on the farm of origin (90% shedding with 10^6 cfu/g) while testing of skin swabs from the same pigs in pre-slaughter lairage revealed increased prevalence (95%) (562). Rectal swab prevalence remained high immediately post-slaughter (76% with 10^7 cfu/g) while pre-chill entire carcase swabs revealed 100% prevalence with an average load of 10^3 cfu per unit area in this study. Final testing of post-chill meat products derived from these same original pigs revealed a prevalence of 49% but a load of only 18 cfu/g. It should be noted that other studies reported lower prevalence: for example a recent study in Ireland (566) reported prevalence in caecal, carcass and pork product samples of 26%, 10% and 15% respectively. A study undertaken in Denmark in 2005 reported 90% prevalence of *Campylobacter* in faeces, 90% prevalence on pre-chilled carcasses, but only 17.5% prevalence on post-chilled ones (563). Finally, an earlier US study of 30 pigs passing through processing found 100% prevalence of rectal carriage, 33% carcasses positive after exsanguination, falling to 0% after de-hairing and polishing, rising to 7% positive after evisceration and rinsing but falling again to 0% after overnight chilling at 2^{o}C (567). Potential contamination points were identified to be leakage of gut contents from the anus during polishing or evisceration or through damage to the gut wall during evisceration, with potential for cross contamination of other carcasses during handling, rinsing or chilling (568). The importance of carcass hygiene practices was supported by a correlation between the prevalence of *Campylobacter*-positive carcasses and the prevalence of carcasses requiring trimming of pleuritis lesions - indicating a potential role for increased operator handling in *Campylobacter* contamination (569). The introduction of blast chilling of carcasses has facilitated marked reduction in prevalence of *Campylobacter* contamination with one study demonstrating reduction form a 56% prevalence on pre-chill carcasses down to 1% following blast-chill (570).

7.1.5 A somewhat lower prevalence of gut carriage of *Campylobacter* species has been reported for cattle and sheep compared to pigs (564). These two species also show a relatively increased prevalence in gut content of *C. jejuni* versus *C. coli*: Milnes et al noted 54.6% prevalence in cattle (of which 81% was attributed to *C. jejuni*) and 43.8% prevalence in sheep (of which 65% was attributed to *C. jejuni*). Less recently, however, prevalence in sheep intestine was estimated at 91% - again with a

dominance of *C. jejuni* isolates (571). Processing of cattle and sheep in the UK involves: transportation, for up to a standard legal limit of 8 hours; lairaging of incoming animals for a maximum of 48 hours; stunning by electricity or captive bolt with killing by exsanguination; followed by de-hiding and evisceration. Evisceration is a 2-step process, as with pigs, in which the carcass is suspended head down while abdominal and then thoracic viscera are removed. Specified risk material must be removed from ruminant carcases, including spinal cord material from bovine carcasses, a process that requires the carcass to be split in two. Carcasses may be rinsed with potable water to remove bone dust prior to chilling at 7^{o}C but legislation requires that visible contamination be removed by trimming rather than washing. A recent study of 98 cattle moving through this process in the USA found a 77% prevalence of *Campylobacter* in faeces collected on farm (10^4 cfu/g) and 82% prevalence on the hides of live animals in lairage (0.9 cfu/unit area) (572). Downstream sampling of these same animals revealed a post-exsanguination rectal sample prevalence of 97% (load 10^5 cfu/g), a pre-chill carcase prevalence of 55% (load 8.7cfu/unit area) and a final prevalence in chilled minced meat of 12% (load 1.1 cfu/g). Contamination points for faecal bacteria including *Campylobacter* include transfer of contamination from the hide or skin during skinning and, as with pigs, leakage of gut content during evisceration with potential for cross contamination of carcases during handling or chilling (568)

Prevalence of *Campylobacter* spp. on red meat

7.1.6 Available evidence, although somewhat dated, indicates a low prevalence of *Campylobacter* contamination on red meat at retail in the UK. A survey commissioned by FSA found an overall prevalence of 0.36% for *Campylobacter* among a total of 5998 samples of beef (0.13%), pork (0.46%) or sheep (0.92%) meat collected in the period 2006-7 (573). Prevalence data for *Campylobacter* spp. detection on red meat among EU Member States, collated by EFSA, also indicates low levels of contamination. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2013 reported available data for red meat at retail by country and red meat type; reports ranged from 0% for countries including Spain and Finland, through 0.44% of 686 pig meat samples in the Netherlands, 0.7% of 430 bovine meat samples in the Netherlands, to 2.1% of pig meat samples in Hungary. Earlier EFSA-collated data across longer

time periods for individual red meat types indicates a similar picture. For pig meat, a mean prevalence of 2.6% was reported based on the period 2007-2009 for 12 member states (Scientific Opinion on the public health hazards to be covered by inspection of meat (swine)), but samples were likely to have been sourced from a combination of sites including intestinal content and carcass swabs. Such data, generated by processors as part of national surveillance and reporting schemes might also suffer from limitations in sampling quality or test sensitivity. As such this EFSA data should be considered in the context of wider sources of such information. Indeed, a higher prevalence was reported elsewhere in Europe. For example, a prevalence of 6.3% was reported for pig meat samples at retail in the UK in the period 2003-5 (564), 10.6% in Poland (574), 15.6% in Ireland (566) and 10.3% in New Zealand (575). Where stated, *C. coli* was the dominant isolate in these studies.

7.1.7 Collated EFSA data on bovine meat prevalence for the period 2008-2011 for samples collected at unspecified stages of processing, among 4 Member States, showed a mean prevalence of 3.9% (0 – 38.5%) with a high degree of variance (Scientific Opinion on the public health hazards to be covered by inspection of meat (bovine)). Other surveys of beef samples at retail indicated a prevalence of 4.3% in the UK (576), 12% in the US (572) and 10.1% in Poland (574) while a Canadian retail beef survey found 0% prevalence by culture and isolation but positive results by PCR (27% for *C. coli*, 14.8% for *C. jejuni*) indicating the potential for viable contamination exists (577).

7.1.8 The reported prevalence of *Campylobacter* spp. on sheep meat in Europe, based on EFSA collated data, was 1.8% (0 – 5.8%) based on 3 member states covering the period 2004 – 2011 (Scientific Opinion on the public health hazards to be covered by inspection of meat (sheep and goats)). However, a survey of retail sheep meat in the UK between 2003-5 (the major EU producer of sheep meat) indicated a higher prevalence of 12.6%)(576) with dominance by *C. jejuni*, while a 32% prevalence was reported for retail sheep meat samples and 44% of retail sheep liver samples in Greece (578)

7.1.9 The relatively high prevalence of contamination of chicken liver by *Campylobacter* spp. has been acknowledged elsewhere. Liver contamination in red meat species was highlighted in a study of retail samples, using an enrichment step,

from Scotland that reported prevalences of 81% (chicken samples), 69% (cattle), 79% (pig), and 78% (sheep) (417). Based on the prevalence of positive samples in the absence of an enrichment step, the same study determined that bacterial loads were greatest in chicken and cattle livers (>25% positive without enrichment), followed by sheep (10%) and pigs (3%).

Interventions

7.1.10 Interventions to control bacterial contamination of red meat can be categorised into: (i) pre-harvest (farm, transport and lairage); and (ii) processing (stunning, killing, evisceration, dressing, chilling, further processing and packaging). The goal of producing safe red meat has been pursued by controlling pathogenic organisms at multiple stages along the 'farm to fork' supply chain. This approach is captured in EU food safety legislation that requires controls along the supply chains (EC Regulation 178/200, EC Regulation 852/2004), beginning with inputs such as feedstuffs, through primary production to slaughter, processing and retail (EC Regulation 853/2004; EC Regulation 854/2004) with extensive use of Hazard Analysis Critical Control Points (HACCP) principles. However, HACCP plans for use in red meat production are typically focused on the control of pathogenic *E. coli* and *Salmonella spp* rather than *Campylobacter spp*. Relatively little data are available on the effect of interventions specifically targeted at *Campylobacter* control along red meat supply chains; rather, research into interventions aimed at reduction of contamination of red meat by faecal organisms has focused more on *Salmonella* in the case of pig meat, and on *Salmonella* and verotoxigenic *E. coli* for cattle and sheep meat.

7.1.11 At farm level a combination of legislation, farm assurance schemes, and good agricultural practices are employed as generic interventions (579) but very little published literature exists relating specifically to *Campylobacter* control at farm level for red meat species. A seven-point plan for reduction of prevalence of pathogenic *E. coli*, *Campylobacter* and *Salmonella* in cattle has been described, based on empirical review of evidence from field studies (580). This recommended "Dry and clean bedding, stable rearing groups, empty and clean water troughs every 2–3 weeks, rodent control, closed herd (or at least closed young stock sections), avoid young stock contact between herds, leave a down-time period between manure spreading on or close to grazing fields before allowing cattle to graze." However, there is other

evidence indicating that some steps currently recommended in legislation or as good hygienic practices such as ensuring visible cleanliness (581), or ensuring as short journey times to slaughter as possible (582), both important measures in limiting *Salmonella* shedding, have limited association to *Campylobacter* contamination after processing. The suitability of an approach based on on-farm interventions to minimise *Campylobacter* contamination of ruminant red meat was considered unlikely to be beneficial, based on its widespread presence in the environment, intermittent shedding without clinical signs of disease and the difficulty of exclusion of the organism even from highly controlled productions systems (579). Similarly, in the case of pigs, alternative production systems such as organic outdoor rearing were associated with diverse populations of *Campylobacter* spp, as was reported for conventional pig production, with a similar bias towards *C. jejuni* over *C. coli* (583) – again highlighting the great difficulty in effectively controlling *Campylobacter* at the farm production stage.

7.1.12 Controls for microbiological contamination at the level of processing were recently reviewed by the European Food Standards Agency (EFSA) in a series of reports giving consideration of the public health hazards to be covered by inspection of meat for bovine animals, sheep and goats, and pigs (Scientific Opinion on the public health hazards to be covered by inspection of meat (bovine animals, sheep and goats, and pigs)). These reports concluded that *Campylobacter* was a low risk organism in terms of transmission to humans *via* red meat. This decision was based on the low prevalence described in collated EU-wide surveys and, importantly, the significant impact of conventional or blast chilling and desiccation in reducing *Campylobacter* survival on the surface of refrigerated red meat carcasses (568, 570). The ACMSF's Second report on *Campylobacter* concluded that legislated existing hygiene procedures for red meat at processing, aimed primarily at controlling *Salmonella* and Shiga toxin associated *E. coli* (STEC), including food chain information, ante mortem inspection (including evaluation of cleanliness of hides), steps to minimize contamination at skinning and evisceration, post mortem inspection and finally chilling were considered effective in controlling *Campylobacter* contamination.

7.1.13 It is, nevertheless, important to note that molecular epidemiological

attribution studies have identified the importance of bovine and ovine reservoirs of *Campylobacter* for human disease, indicating the existence of likely environmental pathways for transmission other than through red meat consumption (Strachan et al 2009).

Conclusions

7.1.14 Red meat presents a low risk for food-borne transmission of pathogenic *Campylobacter* spp. to consumers.

7.1.15 Available evidence indicates that existing process controls, especially chilling of carcasses, provide an effective means for control of *Campylobacter* along red meat supply chains.

7.1.16 The high prevalence of *Campylobacter*, including *C. jejuni*, among red meat livestock on farms combined with existing attribution data indicates that environmental, non-food borne, pathways for human infection likely exist.

Recommendations

7.1.17 Regular structured surveillance for *Campylobacter* contamination of red meat at retail, updated at least every 5 years, would enable on going assessment of changes in this route for human exposure. Such surveillance is justified by widespread carriage of *Campylobacter* among red meat species, the potential for contamination during processing and current reliance on the effectiveness of chilling as a critical control point in reducing final exposure *via* retail fresh red meat.

7.1.18 If processing methods were to change in ways that lead to higher contamination rates and levels then this would be concerning since there might be impact on consumer contamination. Therefore, risk assessment steps for future adaptations to red meat processing methods should routinely take account of *Campylobacter*.

7.1.19 Further research to understand and manage environmental pathways for human exposure linked to primary production of red meat livestock species is justified.

7.2 Other foods including raw/pasteurized milk and fresh produce

Raw/pasteurized milk and milk products

Market Statistics

7.2.1 For raw milk/cream, there are no accurate data available for the UK market on production or sales volumes. In 2010, it was estimated that there were around 100 registered raw cows' drinking milk producers in England and Wales, 27 producers of raw goats' milk, and 3 producers of raw sheep milk. Volumes are difficult to estimate since they are likely to vary according to demand. The sale of raw milk is limited to farm gate sales, farm catering and farmers markets.

7.2.2 For processed milk, the total volume for the UK for the past 12 months was 5,493,424 l, made up of 4,776,291 l pasteurised, 285,764 l filtered, 2234,567 l UHT, and 7,011 l sterilised milk. Other milk types and soya milk constitute the remaining 189,802 litres (AHDB, 2016).

Contamination sources

7.2.3 Milk, including both raw and pasteurised, has been implicated in several outbreaks of campylobacteriosis (see Table 7.4). Raw, unpasteurised milk is the most common vehicle reported in outbreaks of campylobacteriosis (584). The contamination sources for raw milk include faecal matter (from the cattle themselves), wild bird droppings, poorly sanitized milking equipment, milking equipment contamination during repair, human carriers and silent mastitis (283, 377, 388, 585, 586). Of these different potential sources, faecal contamination is considered to be the main cause (371, 587-589) and ruminants are known to constantly shed *Campylobacter* into the environment. In a recent study in Italy (590), there was also good evidence of chronic udder infections contributing to bulk milk contamination. On one of the farms included in this study, segregation of an infected animal resulted in undetectable levels of *C. jejuni* in bulk milk, confirming udder infection as a likely cause. The different steps of processing of raw and pasteurised milk are shown in Fig. 1 below.

7.2.4 The principle cause of contamination in milk is the faeces, on the external surfaces of the udder and teat. Hence reducing faecal contamination of the udder

before milking is a key step, as is good animal husbandry through avoiding mastitis. EU Regulation 853/2004 requires that milking is carried out hygienically by ensuring that the teats, udder, and adjacent parts are clean. Despite best efforts, it is inevitable that some contamination with faecal material will occur, and equipment used for milking, such as suction cups, pipes, buffers and holding tanks may allow contamination to spread more widely. The importance of effective regimes for rinsing, detergent washing followed by disinfection cannot be overstated. The same is true for the steps following milking, as described in Fig. 1. For raw milk, there are no further controls in downstream processing that are designed to, or capable of decontaminating milk.

7.2.5 For pasteurised milk, the main critical control point in processing is the pasteurisation step. This commonly involves high temperature, short time processing where milk is heated to 71.7^{o}C for 15 seconds. Pasteurisers are complex pieces of equipment that must be properly maintained and particular features are essential for their safe and effective operation. These include an effective quality management system in place, equipment servicing at regular intervals, verification of heat exchanger integrity (i.e. no leaks in plates used for heating), flow diversion checks and correct function, verification of holding time at regular intervals and phosphatase testing. More detail of the key steps in milk pasteurisation are provided by Bell and Kyriakides (591). In addition, this text refers to a survey of dairy establishments in the UK between 1999 and 2000 where plants were asked about their compliance to key process control and preventative control measures. The results of this survey indicated that a significant proportion of plants (14-29%) were not applying the measures identified. Several outbreaks associated with raw milk, pasteurised milk and milk products are listed in Table 7.4 below. These focus on outbreaks reported in the UK but also include some outbreaks linked to cheese and cheese products in the USA. The table provides clear evidence that raw milk is a primary source of campylobacteriosis outbreaks in the UK, and also lists some examples of outbreaks caused by pasteurised milk, with a number of these occurring relatively recently, demonstrating that the risks for these products remain significant if there are failures in control measures and pre-requisites, such as good hygiene. In addition to the data shown, in the EU in 2015, 27.3% of outbreaks associated with consumption of ‘milk,

cheese and dairy products' was caused by *Campylobacter*, at a higher frequency than in any other category (592). These outbreaks include 14 caused by raw milk.

7.2.6 Consumption of raw, unpasteurized milk has been the leading cause of campylobacteriosis outbreaks in Europe, the US, and Canada for the past 15 years (92). For raw milk outbreaks, several studies (e.g. (377, 593, 594)) have reported indistinguishable genotypes/almost identical strains of *Campylobacter* spp. from dairy cattle and humans, concluding that dairy cows and calves are likely sources of *C. jejuni* causing human campylobacteriosis. It is also interesting to note that a single or dominant subtype has typically been reported for milk-borne outbreaks where subtyping has been undertaken, demonstrating the value of genomic approaches for detecting these outbreaks. In two investigations, analysis of allele differences between highly related strains shows only three or four polymorphisms (132, 594).

Figure 1 Process flow diagram and key safety considerations for pasteurised milk

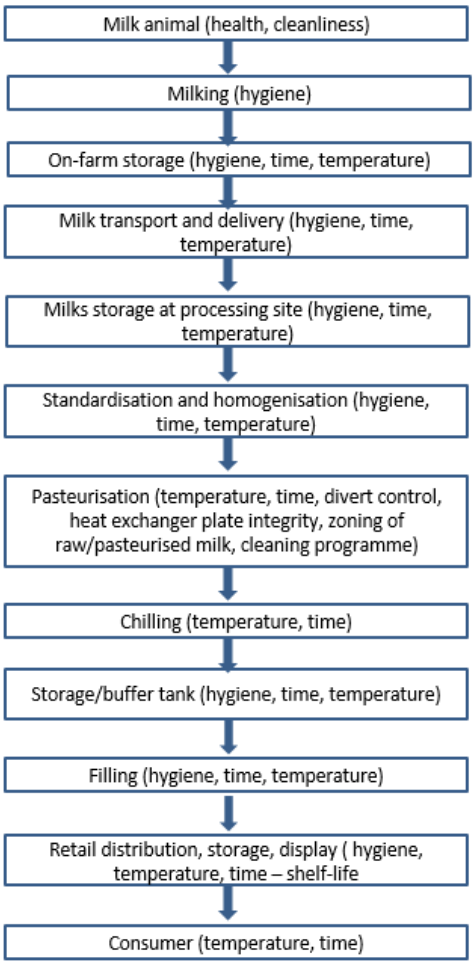


Table 7.4 Outbreaks of campylobacteriosis associated with milk or milk products

Food	*Campylo-bacter* species	Year	Country	No. of cases	Premises	Reference
Raw milk[1]	NR	1978	UK	100	Multiple	(595)
Raw milk	NR	1978	UK	64	Farm	(595)
Raw milk	NR	1978	UK	16	Farm	(595)
Raw milk[1]	*C. jejuni*	1979	UK	148	Multiple	(596)
Raw milk[1]	*C. jejuni*	1979	UK	>75	Private homes	(595)
Raw milk	NR	1979	UK	11	Private homes	(595)
Raw milk	NR	1979	UK	4	School	(595)
Raw milk	NR	1979	UK	14	Institution	(595)
Raw milk	*C. jejuni*	1980	UK	75	Agricultural college	(595)
Raw milk	NR	1980	UK	30	School	(595)
Raw milk	*C. jejuni*	1980	UK	40	Private homes	(595)
Raw milk	NR	1980	UK	7	Private home	(595)
Raw milk	NR	1992	UK	72	Outdoor festival	(597)
Raw milk	NR	1993	UK	22	Outdoor festival	(597)
Raw milk	NR	1994	UK	23	Farm visit	(597)
Raw milk	NR	1995	UK	35	RAF base	(597)
Raw milk	NR	1996	UK	5	Farm	(598)
Raw milk	NR	2002	UK	3	Farm	(599)
Raw milk	NR	2007	UK	9	Farm	(591)
Raw milk	NR	2016	UK	56	Farm	(600)
Pasteurized milk	*C. jejuni*	1979	UK	2500	School	(601)
Pasteurised milk	NR	1992	UK	110	Doorstep delivered pasteurised milk	(598)
Pasteurized milk	*C. jejuni*	1995	UK	110	Dairy	(602)
Pasteurized milk	*C. jejuni*	1992	USA	23	Farm	(603)
Pasteurized milk	*C. jejuni*	1990	UK	32	Consumer homes – bottles attacked by birds	(604)
Pasteurized milk	*C. jejuni*	2011	UK	27	Dairy	(220)
Dairy cottage cheese	*C. fetus*	1992	USA	13	NR	(605)
Cheese made with	*C. jejuni*	2000	USA	18	Private home	(606)

unpasteurized milk						
Custard made with UHT milk[2]	*C. jejuni*	2003	Spain	81	School	(607)
Mexican soft cheese (queso fresco)	NR	2003	USA	11	Pot luck dinner	(608)
Raw milk cheese	*C. jejuni*	2006	USA	58	Homemade	(606)
Raw milk cheese	*C. jejuni*	2007	USA	16	Local fair	(606)
Soft cheese from unpasteurised milk	*C. jejuni*	2007	USA	67	Local community fair	(608)
Mexican raw milk cheese (queso fresco)	*C. jejuni*	2009	USA	10	NR	(606)
Mexican raw milk soft cheese	NR	2010	USA	1	Door-to-door sales	(608)
Pasteurised whole milk cheese curds	*C. jejuni*	2010	USA	3	NR	(609)
Cheese curds	*C. jejuni*	2012	USA	2	NR	(609)
Sheep cheese	*C. fetus*	2015	Netherlands	5	NR	(592)

[1] – Weather conditions prevented pasteurisation of milk

[2]- Cross contamination suspected as cause

7.2.7 The epidemiology of thermophilic *Campylobacter* in dairy herds remains poorly understood and several variables such as herd size, and herd type, season, climate, animal age, geography, diet and husbandry practices are thought to play a role.

7.2.8 A recent study (297) reports a distinct temporal trend in faecal shedding in herds in Italy, with two prevalence peaks between November and December, and between May and July. The reasons for this remain unclear and a number of factors were ruled out as a likely cause of the seasonality trends. Due to differences in housing systems and climate conditions, these results may not be representative of other countries but seasonality of faecal shedding may play an important role. Following a large outbreak in the US, an investigation concluded that a single production run did not explain all the identified illnesses, suggesting repeated

contamination, despite minimal deficiencies being identified during inspections. In addition, the microbiological tests and standards for raw milk in the US do not ensure that the raw milk is free of pathogens (585). In a large outbreak in the US in 2014, the dairy implicated submitted samples every 4 weeks and the counts reported continually yielded acceptable results before and throughout the outbreak investigation (352). In the UK, studies following two milking herds reported that *Campylobacter* can produce symptomless and persistent infection or colonisation in milking herds without any detectable contamination in milk, that prevalence in faecal excretion varies considerably with season, and that infection may be more easily established in young animals possibly persisting into adulthood (595).

7.2.9 Consumption of raw milk is also reported to play a major role in sporadic cases of campylobacteriosis. In Minnesota, 6% (407 cases) were reported between 2001-2010 and raw milk consumption was estimated to have caused more than 12,000 cases during this period (610).

7.2.10 These outbreaks continue to serve to demonstrate the importance of pasteurization and the need to educate consumers to highlight the risk of serious illness that may result from consumption of unpasteurised dairy products, particularly for 'high risk' groups such as pregnant women, immunocompromised individuals and young children. There is a belief amongst a growing number of consumers that pasteurization diminishes the health and nutritive benefits of raw milk, despite the known risks (611). In a comparison of non-pasteurized milk foodborne disease outbreaks reported in the US from 2007 to 2012, Mungai et al (354) reported that the number had increased from 30 between 2007-2009 to 51 during 2010-2012, with most of these caused by *Campylobacter* spp. The authors point towards increasing demand for raw milk and state legislatures relaxing restrictions on the sale on non-pasteurized milk.

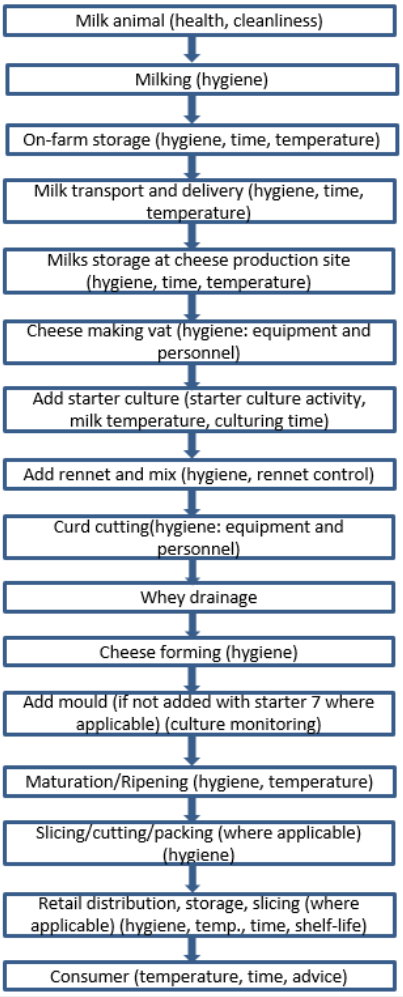
7.2.11 In five of the outbreaks reported in the UK linked to pasteurised milk (354) reported that the number had increased from 30 between 2007-2009 to 51 during 2010-2012, with most of these caused by *Campylobacter* spp. inadequate or faulty pasteurization processes were identified as the cause. In a number of these cases, information gathered from investigations indicated that recurrent pasteurisation failures were occurring but only single outbreaks were identified. In the large

outbreak reported by Jones et al. (601), the investigation suggested that inappropriate operation of the bypass valve system may have resulted in bulk milk passing through the plant without being processed. With pasteurization, there are measures that can be put in place to verify that pasteurization processes are effective and monitoring systems, such as the phosphatase test, that indicate when processes have failed (see above).

7.2.12 Cheese and cheese products (such as *queso fresco*) made from unpasteurised milk have also been identified as the cause of a number of outbreaks of campylobacteriosis in the United States (606, 608). It is commonly thought that *Campylobacter* lacks the ability to adapt to and survive in harsh environmental conditions such as high NaCl/reduced water activity, reduced pH and low temperatures, and several studies clearly demonstrate die-off during ripening processes used for hard and semi-hard cheeses. However, epidemiological investigations provide evidence that some cheeses support survival of organisms that remain infectious and capable of causing disease. Outbreaks linked to cheeses made using unpasteurised milk are infrequent, in comparison to outbreaks caused by raw, unpasteurized milk. The processes used in the production of cheeses made from unpasteurised milk show wide variability and while many of these involve a fermentation step, others do not. For example, *queso fresco* is a soft cheese which is processed without fermentation and is marketed rapidly after production. The process used for a typical cheese made using unpasteurized milk is shown in Fig. 2 below.

7.2.13 Some risk assessment studies have been carried out on risks of campylobacteriosis associated with consumption of raw milk. In a risk assessment by FSANZ (612), a sensitivity analysis on factors having the greatest impact on contamination of bulk milk identified degree of teat soiling and within-herd prevalence as the two most important factors, with herd size having no influence and teat cleaning efficiency having only a weak influence.

Figure 2 Process flow diagram and key safety considerations for cheese made from unpasteurised milk



Prevalence of *Campylobacter* spp. (data on prevalence and levels; types; country; years)

7.2.14 Table 7.5 summarises studies reporting on the incidence of *Campylobacter* spp. in some raw milks, demonstrating that prevalence can be as high as 12% in raw milk from bulk tanks, in Europe, and even higher in some other regions e.g. 27% in China. In the UK, Stanley and Jones (584) reported an incidence of between 3.8 and 8.1%, and Robinson and Jones (595) refer to an outbreak in 1978 in the UK where 10% of the milking herd were found to be excreting *Campylobacter*. Although results from these studies suggest that contamination is uncommon, it is clear from epidemiological evidence that raw milk poses a significant risk for campylobacteriosis and other infectious diseases, and as such, must be considered a 'high risk' food. In addition, *Campylobacter* is able to survive in refrigerated milk for up to 3 weeks (613) and although it is not able to multiply in the milk, is still capable of causing infection. A key point to consider with all surveys reporting on the prevalence of pathogens in foods is that non-detection does not mean absence, and actual prevalence may be higher than observed in the studies, particularly when the limited sampling plans employed do not allow for a true estimation of the variability and distribution to be determined.

7.2.15 Several surveys that have investigated the prevalence of *Campylobacter* spp. in cheese made from raw milk report absence in products tested, and this is consistent with epidemiological information that has identified particular types of cheese that pose a risk. Many other types of cheese, particularly semi-hard and hard ones, do not support survival and will promote rapid die-off when certain conditions prevail, such as reduced pH and increased NaCl concentrations /reduced water activity. For example, in a survey of 41 raw milk aged (minimum of 60 days) cheeses (i.e. not including cheeses such as *queso fresco* cheese) from retail outlets, no *Campylobacter* were recovered (614). In the European Union in 2013, only one sample tested positive out of a total of 428, from various sources including retail and processing plants (615). The US FDA requires that 60-day aging should be applied to improve the microbiological quality of cheese made from unpasteurized milk (616) and this appears to be effective for more sensitive microbiological targets such as campylobacters but may not be as effective for controlling other pathogens such as *E. coli* O157:H7.

Table 7.5 Incidence of *Campylobacter* spp. in some raw milks

Food	% positive	Year	Country	Reference
Raw cow's milk	0	1996-1996	UK	(617)
Raw cow's milk	2	1996-1997	UK	(617)
Raw goats, sheep and buffalo milk	0.5	1997-1999	UK	(617)
Raw cow's milk intended for pasteurization	0.8	1999-2000	UK	(617)
Bulk tank milk	1.6	2001-2002	Ireland (incl. Northern Ireland)	(38)
Bulk tank milk	9.2		USA	(618)
Bulk tank milk	0.3	2007-2008	New Zealand	(619)
Bulk tank milk	2.0		USA	(618)
Raw Milk from automatic vending machines	2.1	2011	Italy	(620)
Bulk milk tank	0.6	2011-2012	New Zealand	(621)
Raw milk at retail	0-12.5	2013	Germany	(489)
Raw milk at retail	0-0.61	2013	Italy	(489)
Bulk tank milk	12	2014	Italy	(590)
Raw milk intended for manufacture of raw or low heat-treated products	16.67	2014	Spain	(615)
Raw milk	0-5.26	2014	Germany	(615)
Raw milk intended for direct consumption, from farm	2.56	2014	Estonia	(615)
Raw milk	0-55.56	2015	Italy	(592)

Goats' milk outbreak – see Dairy Australia & NZ report.

7.2.16 Considering other dairy products, there have been 2 campylobacteriosis outbreaks in the US associated with home-made ice-cream (622). These serve to

demonstrate that freezing cannot be relied upon to eliminate *Campylobacter* contamination in foods, even though some studies report reductions in numbers.

Raw fruits and vegetables

Market Statistics

7.2.17 In the past 30 years, the fruit and vegetable market has been one of the fastest growing sectors of all products, and between 2002-2004, there were 500 million tons of fruit and 800 million tons of vegetables produced globally. Annual growth in production of vegetables was around 4.2%, approximately double that of fruits between 1980-2004. The volume traded as fresh is >5% total production. The EU and USA are among the largest importers and exporters. In the EU, foodborne outbreaks associated with vegetables and fruits has increased from 4.4% in 2009 to 10% in 2010. The Netherlands, UK and Belgium are the leading direct importers of fresh fruit and vegetables from developing countries, with UK importing more than 2 million tonnes of fresh fruit from developing countries and almost 1.5 million tonnes from the EU, and approx. 0.2 million tonnes and over 2 million tonnes of fresh vegetables from developing countries and the EU respectively. In a report from DEFRA (2016), home produced vegetables were worth £1.3 billion in 2015, showing a 3.9% increase on 2014, driven mostly by increased sales of carrots, mushrooms and cabbages. Field vegetables were worth £884 million and protected vegetables, £393 million. For fruit, the value of home produced rose to £695 million, increasing by 9.6% compared to 2014. Home-produced vegetables and fruit contributed 57% and nearly 18%, respectively, of total UK supply in 2015. Overall, supply of vegetables and fruit has increased by 12% between 2007 and 2015.

Contamination sources

7.2.18 Raw fruits and vegetables may be contaminated with faecal matter and this is a potential source of campylobacteriosis. Poultry manure is sometimes used for the cultivation of fresh produce in some regions (623) and therefore consumption of this produce when eaten raw may to lead to exposure to *Campylobacter* spp. Irrigation water may also be a source, together with domestic or wild animals and human handling. Contamination may also occur during harvesting, processing (e.g. wash/rinse water, cutting, ice), packaging and distribution, or at retail level (624). The

main steps involved in growing, harvesting, and processing of fresh produce are shown in Fig. 3.

7.2.19 In an investigation of unpacked and packed fruit and vegetables in the Netherlands, Verhoeff-Bakkenes et al. (625) reported that 0.36% of packed produce were positive for *Campylobacter* spp. compared to 0.07% for unpacked products. Some studies report high prevalence rates and this may be due to differences in hygienic practice in some regions. In the UK, three studies (626) (627, 628) *Campylobacter* spp. were not detected and in another (629), 22% of samples were found to be positive. The important role of hygienic handling is demonstrated in a UK-based study (630) that looked at outdoor market samples and produce in supermarkets, where 9 out of 533 were positive in samples from the market and all 1031 supermarket samples were negative. Even though the contamination rates may be low relative to contamination rates reported for other foods, such as poultry and raw milk, the high consumption patterns and likelihood of these products being consumed raw means that consumption of these products may be a risk factor for campylobacteriosis.

7.2.20 Table 7.6 provides a summary of campylobacteriosis outbreaks associated with fresh produce. In a review of outbreak in the US, Taylor et al. (631) reported 5% of 262 outbreaks from 1997 to 2008 linked to produce e.g. leafy greens (3), melons (1), tomatoes (1), sweet potato, cucumber, and strawberries. These outbreaks were responsible for 565 cases. Gardner et al. (370) investigated a community-wide outbreak in Alaska and concluded this was caused by consumption of locally grown peas contaminated with faeces from Sandhill cranes grazing in the farms pea fields. As a consequence, the farmer was advised in install food-grade-water-utility lines and a chlorine injector, and make other changes to minimise the risk of cross-contamination and adhere to FDA-recommended handling practices (632). In a review of campylobacteriosis outbreaks in the US between 1990 and 1999, (633) reported that produce was associated with more cases of illness than any other food source, and second to dairy products in the total number of outbreaks.

Table 7.6 Outbreaks of campylobacteriosis associated with fresh produce and salads

Food	*Campylo-bacter* species	Year	Country	No. of cases	Premises	Reference
Salad[1]	Lettuce	1980	USA	41	Summer camp	(634)
Salad	*C. jejuni*	1984	Canada	330	University cafeteria	(635)
Cantaloupe melon	*C. jejuni*	1985	USA	16	Various	(636)
Salad	*C. jejuni*	1987 -96	Japan	NR	School lunch	(637)
Tuna salad[1]	*C. jejuni*	1998	USA	79	Summer camp	(638)
Lettuce[1]	*C. jejuni*	1996	USA	14	Restaurant	(639)
Mixed salad containing ham and feta cheese	*C. coli*	1995	Belgium	24	School	(640)
Lettuce salad	NR	1996	UK	16	Hotel	(641)
Salad items		1996				(633)
Cucumber	NR	1996	Australia	78	Training facility	(642)
Seasonal leaves and tomato salad	*C. jejuni*	1997	UK	12		(643)
Lettuce	NR	1998	UK	NR	Restaurant	(641)
Lettuce		2000	UK	18	Restaurant	(641)
Pasta salad, orange juice	*C. jejuni*	2001	UK	30	Canteen	(641)
Potato salad	*C. jejuni*	2001	USA	24	Camp	(644)
Potato salad	*C. jejuni*	2001	USA	16	Buffet at catering hall	(644)
Fruit salad	*C. jejuni*	2001	USA	14	Picnic	(644)
Guacamole	*C. jejuni*	2002	USA	50	Various	(644)
Tuna salad, green salad, pasta salad	*C. jejuni*	2002	USA	136	Prison	(644)
Potato salad, baked beans	*C. jejuni*	2005	USA	14	Picnic	(644)
Caesar salad	*C. jejuni*	2005	USA	4	Restaurant or deli	(644)
Water melon	*C. jejuni*	2006	USA	15	Picnic	(644)

Raw peas	*C. jejuni*	2008	USA	132	Community-wide	(370)
Pasta salad	*C. jejuni*	2015	UK	33	Restaurant	(645)

[1]Cross-contamination during preparation or poor food storage practices or infected food handler as cause

7.2.21 In the UK, guidance for producers of fresh, ready-to-eat produce has been developed by the Chilled Food Association (CFA) and focuses on appropriate field controls to minimise the risk of contamination by zoonotic organisms, including controlled use of organic waste (e.g. no raw farm yard waste), irrigation water quality and hygiene for food handlers, in addition to controls in preparation and further handling. These guidance documents include Microbiological Guidance for Growers and Water Quality Management Guidelines (see http://www.chilledfood.org/publications/).

7.2.22 The 'safe sludge matrix' was developed in the UK to provide guidance on the use of all applications of sewage sludge to agricultural land, and consists of a table of crop types, together with clear guidance on the minimum acceptable level of treatment for any sewage sludge-based product which may be applied to that crop or rotation. Untreated and conventionally-treated sludges are not permitted to be used on fruit, salads, vegetables or horticulture. For enhanced treated sludges, which deliver a 6-log reduction in pathogens such as *Salmonella*, a 10-month interval applies between application and harvesting.

7.2.23 Good agricultural practice is the application of quality assurance and management at the farm level. GAP guidance documents have been produced by FDA/USDA and are entitled "Guide to Minimise Microbial Food Safety Hazards for Fresh Fruits and Vegetables" (646) and "Guide to Minimise Microbial Food Safety Hazards of Fresh-cut Fruits and Vegetables" (647). The first of these provides recommendations for growers, packers, and shippers to use good agricultural and good manufacturing practices in areas where they have control to prevent or minimise microbial food safety hazards in fresh produce. This guidance is based on 8 basic principles and practice. The second guidance document primarily addresses microbiological hazards and appropriate control measures for these although some chapters also discuss physical and chemical hazards. The key areas covered in the 2nd guidance document are: (i) personnel health and hygiene; (ii) training; (iii)

building and equipment; and (iv) sanitation operations. In a response to the increasing number of outbreaks linked to fresh produce, FDA (648) has implemented new food safety rules for fresh produce to update previously published guidelines. The FDA Food Safety Modernization Act (FSMA) Produce Safety rule sets out science-based minimum standards for the safe growing, harvesting, packing and holding of fruits and vegetables grown for human consumption. This identifies key requirements for agricultural water, biological soil amendments, sprouts, domesticated and wild animals, worker training and health and hygiene, and equipment, tools and buildings.

7.2.24 Other organisations have also provided guidance on GAP, to establish 'best practice'. The Food and Agriculture Organisation (649) advocates a non-prescriptive method that considers the environmental, economic and social sustainability of farm production and post-production processes for production of safe and quality foods. This guidance is based on 10 elements of agricultural practice, many of which are shared with the 8 principles identified by the FDA/USDA. These 10 elements are: soil; water; crop/fodder production; crop protection; animal production; animal health and welfare; harvest and on-farm processing and storage; energy and waste management; human welfare, health and safety; wildlife and landscape. Although it may appear that aspects of animal production and welfare may have no relevance to fresh produce production, this is not necessarily the case. Many of the microbiological hazards that have caused disease and have been linked with fresh produce have animal reservoirs and farm animals are major sources of these agents. Clearly, fresh produce producers must take account of these sources and put in place procedures that will minimise the possibility of these hazards contaminating the produce they are growing.

7.2.25 More recently, GLOBALGAP and the Safe Quality Food (SQF) programme in the US have agreed to a harmonisation of GAP and HACCP-based approaches for food safety management at the farm level (650). GLOBALGAP has identified a number of control points and compliance criteria for fruit and vegetables that address many of the aspects that would be covered by prerequisite programmes. These points and criteria cover soil and substrate management, irrigation, harvesting and produce handling. Other examples where trade associations and retailers are

driving the food safety agenda through development of their own standards are described by Monaghan (651)

Prevalence of *Campylobacter* spp. in fresh produce

7.2.26 Salad vegetables have been reported to be the 2nd highest risk factor for *Campylobacter* infection, after poultry (652). It is a common hypothesis that the outbreaks of campylobacteriosis involving fresh vegetables come from cross-contamination during food preparation (652, 653). A retrospective cohort study involving analysis of data from a questionnaire sent to 2981 sufferers of sporadic campylobacteriosis in the community in Cardiff (652), multivariate analysis was used to identify the main risk factors used. The authors concluded that salad most likely gets cross-contaminated during food preparation due to the finding they observed that the association was specific to items such as tomatoes and cucumber that require extensive handling during preparation, rather than with lettuce or salads bought pre-prepared. Other incidents establishing a link between cross-contamination of produce with cases of campylobacteriosis include a 3-month long outbreak in the UK linked to cucumber served at a salad bar (642) and an outbreak in the US linked to salad prepared by a food-handler suffering from campylobacteriosis (634). A review of outbreaks in England and Wales (654), cross-contamination was identified as the most frequently identified contributory factor. Cross-contamination was identified as the cause of a salad-associated outbreak in Japan (637). Nevertheless, there is evidence that *Campylobacter* spp. may be present on raw vegetables and fruit at the retail level prior to food preparation (655); (656-658) (38, 629). Karenlampi and Hanninen (659) reported an average prevalence level of 0.42% on vegetables. Further details of some of these studies are shown in Table 7.7.

7.2.27 The implications for prevalence levels of *Campylobacter* on fresh vegetables and fruits sold at retail in the Netherlands was investigated by Verhoeff-Bakkenes et al. (625). The study concluded that this would lead to an estimated number of 530,000 cases per year for the whole Dutch population. In a more recent risk assessment, Pielaat et al. (660) predicted 170,000 cases of illness in the Netherlands caused by mixed salads. This discrepancy was reported to be due to differences in the dose-response models used in the two studies, where the beta-

Poisson as used by Verhoeff-Bakkenes *et al*. (625) is thought to overestimate the number of cases at low doses (661).

Table 7.7 Prevalence of *Campylobacter* on vegetables and fruit.

Food	% positive	Country	Reference
Mushrooms	1.5	USA	(656)
Lettuce [1]	1.2	Canada	(630)
Parsley [1]	0.6	Canada	(630)
Cabbage[1]	0	Canada	(630)
Carrots [1]	0	Canada	(630)
Celery [1]	0	Canada	(630)
Cucumber	0	Canada	(630)
Green onion [1]	0.6	Canada	(630)
Potatoes [1]	0.7	Canada	(630)
Spinach [1]	1.1	Canada	(630)
Radish [1]	1.1	Canada	(630)
Lettuce	0	UK	(662)
RTE grated vegetables	1.3	France	(657)
Spinach & Fenugreek	3.7	India	(658)
Fresh mushrooms	0.9	Ireland (incl Northern Ireland)	(38)
Vegetables/salad/sandwiches	0	Ireland (incl Northern Ireland)	(38)
Salad vegetables	23-68%[2]	Malaysia	(655)
Fresh produce	0	Canada	(663)
Leafy greens	0	Canada	(664)
Leafy vegetables	0.36	Netherlands	(625)
Fruit crops	0.17	Netherlands	(625)
Root crops, cabbage, mushrooms, onion, garlic	0	Netherlands	(625)
Stem and sprout crops	2	Netherlands	(625)
Mixed salads/vegetables	0.2	Netherlands	(625)
Vegetable-fruit mix	0.63	Netherlands	(625)
Fruit	0	Netherlands	(625)
Mixed fruit	2	Netherlands	(625)
Market produce	0	Canada	(665)
Endive	0.83	Netherlands	(666)
Oak tree lettuce	2.7	Netherlands	(666)
Strawberries	0	Belgium, Brazil, Egypt, Norway, Spain	(667)

Leafy greens	3.3	Belgium, Brazil, Egypt, Norway, Spain	(667)
Leafy vegetables and leafy herbs	0	Canada	(668)

[1]Positives only from farmers markets where study also included supermarket samples
[2]As detected by PCR, where *Campylobacter* spp. were recovered from 18% of these PCR-positive samples

Drinking water

7.2.28 *Campylobacter*-contaminated water is a common source of outbreaks of campylobacteriosis. In a review of waterborne outbreaks in Canada between 1974 and 2001, 24% (150 out of 288) were attributed to *Campylobacter (669)*. Drinking water originating from storage tanks, community supplies and bottled water have been linked to outbreak of campylobacteriosis. In an outbreak in the UK in 1981, 257 cases of illness were reported in a school that sourced water from a borehole, which was stored in an open-top tank. It is thought that the water became contaminated from faeces of birds or bats, and in this case may well have occurred following maintenance work that is likely to have dislodged debris and dust around the storage tank. Other outbreaks are shown in Table 7.8. A number of these outbreaks from community groundwater supplies were attributed to chlorination failures or heavy rainfall. In most cases, when water has been sampled, *Campylobacter* have not been detected and this is likely due to the lag between illness reporting and contaminated water remaining in the water system, or possibly due poor recovery of surviving cells. Bottled water has also been linked to cases of illness, with one outbreak resulting in 106 cases of illness in US troops that had consumed contaminated bottled water in Greece in 1997. Epidemiological investigation pointed to bottled water as the most likely cause although other risk factors identified also included canteen food and drinking of unpasteurised milk. One other outbreak is also cited by Evans et al. (652). The most common probable causes of campylobacteriosis linked to community water supplies are cross-connections and water treatment breaks resulting from sewage contamination, or heavy rainfall (670). Although these waters may be treated by chlorination or other treatment processes, these will not be effective when contamination arises from a cross-connection in the distribution system or when contaminated water enters the system.

Table 7.8 Outbreaks of campylobacteriosis caused by drinking water

Water source	*Campylobacter* species	Year	Country	No. of cases	Premises	Reference
Water from cold storage tank	*C. jejuni*	1981	UK	257	Various	(73)
Community groundwater supply[1]	*C. jejuni*	1983	USA	865	Various	(671)
Community surface water supply[2]	*C. jejuni*	1984	Norway	680	Various	(672)
Non-community ground water	*C. fetus*	1985	Finland	35	NR	(673)
Community groundwater supply		1985	Canada	241	Various	(674)
Non-community ground water	*C. jejuni*	1986	Finland	96	NR	(673)
Community surface water and groundwater[2]	NR	1986	New Zealand	19	Various	(675)
Community surface water supply[1]	*C. jejuni/coli*	1988	Norway	330	Various	(676)
Water from local springs	*C. jejuni*	1990	New Zealand	44	Camp	(677)
Non-community spring water[2]	*C. jejuni*	1993	UK	267	School	(73)
Community groundwater	*C. jejuni*	1995	Denmark	2400	Various	(678)
Water from dispenser, rain water as source	*C. jejuni*	1997	Australia	23	Staff restaurant	(679)
Bottled water	*C. jejuni*	1997	Greece/USA	106	Army training exercise	(680)
Municipal groundwater	*C. jejuni*	1998	Finland	2700	Various	(681)
Community groundwater	*C. jejuni*	1998	Switzerland	1607	various	(682)
Well water or beverages made with well water	*C. jejuni*	1999	USA	>30	County fair	(683)
Town water supply[2]	*C. jejuni*	2000	Canada	>100	Various	(684)

Tap water from groundwater local community supply[1]	*C. coli*	2000	France	>200	Various	(685)
Community groundwater[2]	*C. jejuni*	2000	Finland	400	Various	(686)
Community supply[2]	*C. jejuni*	2000	UK	281	Various	(687)
Community groundwater	*C. jejuni*	2001	Finland	50	Various	(686)
Community groundwater	*C. jejuni*	2001	Finland	1000	Various	(686)
Community groundwater[2]	*C. jejuni*	2004	Finland	3	Various	(670)
Community groundwater	NR	2007	Norway	15	Various	(688)
Community groundwater	NR	2007	Finland	8453	Various	(689)
Untreated groundwater	*C. jejuni, C. lari, C. coli*	2007	Denmark	140	Various	(690)
Community water supply	*C. jejuni*	2008	Switzerland	126	Various	(691)
Rural distribution system[1]	*C. jejuni*	2009	Greece	36	Various	(692)
Community water supply[2]	*C. jejuni*	2010	Denmark	176	Various	(693)

[1]Chlorination failure
[2]Heavy rainfall
[3]Total number of people exposed

7.2.29 The number of sporadic *Campylobacter* infections caused by contaminated drinking water may be significantly underestimated since not all infections lead to overt illness (694) and mild forms of disease are quite common and go unreported (695, 696). Epidemiological links are particularly difficult when private wells and surface water are used. However, in Canada, one study examined the association between drinking water, agriculture and 2992 cases of sporadic campylobacteriosis, and found that the odds of campylobacteriosis were higher for individuals sourcing drinking water by private wells than municipal water systems (697).

Interventions and pre-requisites

7.2.30 Prevention of outbreaks associated with naturally sourced water can be achieved through chlorination, where low residual levels are known to be effective in destroying *Campylobacter* spp. but control of contamination from pests, dust, dirt, and other debris is also essential, together with effective cleaning regimes for storage tanks and pipework. Bottled water has the advantage of having other technologies available to destroy contaminating microorganisms that may be present in the source water used. These include chlorination, as for borehole water, and also include UV treatment, filtration and ozonation, prior to bottling.

7.2.31 The main interventions and pre-requisites are as follows:

- Water security – prevention on contamination from external sources such as heavy rainfall, surface run-off, sewage contamination, cross-connection problems, lake infiltration, animal faeces;
- Effective filtration and decontamination/disinfection regimes such as chlorination, UV treatment at treatment works and where water is sourced from untreated local supply.

Prevalence of *Campylobacter* spp. in water

7.2.32 A number of studies have reported on the presence of *Campylobacter* in water, particularly environmental water and these are mostly qualitative in nature although there a few that describe levels found. The key factors that influence survival of thermophilic *Campylobacter* in water include: light; temperature; biotic reactions; oxygen concentration; and nutrients (698). As with other environments, survival appears to be enhanced by low temperature, absence of sunlight and low numbers of indigenous microflora. In sewage effluent, numbers as high as 100,000 CFU/l have been reported in Germany (699). Of the different species, *C. jejuni* is most commonly isolated from surface waters and occurrence is associated with sewage discharge but *C. coli* and *C. lari* are also found, particularly where there is agricultural run-off and large flocks of water fowl are present. The presence of high levels in some waters and epidemiological evidence for waterborne campylobacteriosis point strongly to inadequate treatment of drinking water systems as a cause of illness and emphasise the importance of effective disinfection of these systems.

Table 7.6 Prevalence of *Campylobacter* in environmental water.

Water source	Levels reported (CFU per litre)	Country	Reference
River system	0-2300	UK	(700)
Stream water	0-595,000	USA	(701)
Fresh and marine surface water	136-4,600,000	USA	(702)
Bathing waters	0->100	Finland	(703)
Surface waters	0-1000	South Africa	(704)

Seafood

7.2.33 Contaminated shellfish have been implicated as a vehicle causing cases of campylobacteriosis. Harvesting shellfish from *Campylobacter*-contaminated waters has been proposed as the most likely cause of these cases (705). In addition, Federighi et al. (657) reported 5 out of a total of 660 oyster samples being positive for *Campylobacter*, and Wilson and Moore (1996) reported 2%, 8% and 24% contamination rates for *C. jejuni*, *C. coli* and *C. lari* respectively, in shellfish sampled in the UK. A more recent study for retail foods sold in Ireland reported 2.3% of oyster samples containing *Campylobacter* (38). Taylor et al. (631) reported 1.9% of campylobacteriosis outbreaks linked to consumption of seafood in the USA, between 1997-2008, resulting in 276 illnesses.

Conclusions and recommendations

7.2.34 Considering the other foods described in this section, the one that has caused most outbreaks of campylobacteriosis both in the UK and more widely, is raw milk. The relative contribution to the total number of campylobacteriosis cases, however, remains very low, due the low exposure in the population i.e. the low numbers of consumers drinking raw milk. Several factors including symptomless carriage and persistent colonization in dairy herds, variability in shedding, likelihood of repeated contamination events and lack of an intervention step that will destroy contaminating organisms all lead to the conclusion that consumption of raw milk can be hazardous for consumers. This is not only relevant to *Campylobacter* but also

includes a number of other infectious agents than can be associated with raw milk. It is recommended that opportunities are taken to remind consumers of the risks associated with consumption of raw milk. Absence of effective CCPs for raw milk and some cheeses made from unpasteurised milk require the highest standards of hygiene. Farmers that sell raw milk must follow good hygienic practices and ensure that they minimize the risk of contaminating raw milk through effective cleaning and disinfection of equipment used for harvesting and storing raw milk. Considering the limited epidemiological data from outbreaks in the UK, the risk for raw milk does appear to have decreased in recent years in the UK.

7.2.35 Pasteurised milk poses an extremely low risk of campylobacteriosis, as shown by the small number of outbreaks in the UK. The controls, validation and verification systems available for pasteurization processing equipment are reliable if properly maintained and applied. For example, testing of pasteurised milk for phosphatase activity is a simple and effective verification procedure and equipment can easily be set up to divert product that has been underprocessed. No additional measures are recommended here.

7.2.36 Cheese made using raw milk, fresh produce, seafood and water also pose an extremely low risk of infection with *Campylobacter*, due primarily to the low likelihood of survival of the organism in these foods and the low contamination rates reported. Fermentation conditions for semi-hard and hard cheeses will promote die-off of *Campylobacter* spp. Good Agricultural Practice and Good Hygienic Practice in primary production, including manure treatment/quarantine before application, good quality water used for irrigation/pesticide reconstitution, hygiene during harvesting and transport are all well established preventative controls. Also important are policies for workers to prevent those with gastrointestinal infection symptoms contaminating produce either during harvesting or processing/handling. It is important that seafood is sourced from areas that are free from contamination from non-disinfected wastewater discharge, and effective depurination measures for shellfish are well described and quite simple to apply. No further measures are recommended for these foods since good agricultural, hygienic and manufacturing practices will effectively manage the risk for these foods and chlorination treatment will manage the risk for drinking water. Although there has been a 10% increase in consumption of fresh produce in the UK between 2007 and 2015, there has been no

increase in the frequency of outbreaks associated with these foods. This does not rule out an increase in sporadic cases and cases of illness that may be linked to fresh produce but have not been identified as such, so we advise that cases are monitored closely in the future and whole genome sequencing used for sporadic cases to determine if these may be linked to a common source.

7.2.37 For the future, in relation to risks associated with consumption of raw milk, interventions to decrease herd prevalence of *Campylobacter* would require more understanding of the main factors influencing this. In addition, segregation of infected cows for milking (raw milk) and regular checks for animal health would help to reduce prevalence and reduce the risk of raw milk becoming contaminated. However, this is unlikely to be 100% successful and there is likely to be some remaining risk, therefore judgements would need to be made on the value of any such research.

Chapter 8: People's attitudes and behaviours regarding risk (includes consumers, caterers, farmers and the food processing industry)

Introduction

8.1 The wider current risks posed by *Campylobacter* within the human food chain are discussed in other parts of this report. This chapter focuses on the knowledge, attitudes and behaviour relating to these risks among people involved in the production and consumption of foods likely to contain *Campylobacter*. That includes farmers and others working in either primary production or food industry processing, people working in the catering industry and consumers. The latter are the main focus but a number of recommendations are also made for the catering industry. Equipping such individuals with appropriate knowledge, attitudes, motivation and food-handling practices protects them and others from campylobacteriosis and its more severe sequalae.

8.2 In assessing the relative importance of public attitudes and people's domestic practices in controlling *Campylobacter*, it is recognised that most incidents of infection with these bacteria are sporadic which makes accurate source attribution difficult (706). Recent studies have clarified that, in overall terms, most human exposure to *Campylobacter* is related to direct contact with animals, raw or undercooked meat, unpasteurised milk and environmental reservoirs such as natural bodies of water (343). As noted in a previous FSA report (490), contact with raw/undercooked poultry, especially chicken, is the main source of human infection in the human food chain. A number of attribution studies published since then have reconfirmed the importance of raw chicken in relation to human campylobacteriosis (146, 411, 419, 420). These studies have also clarified the less significant, but still important, role of other raw meat products as sources of human infection with this organism.

Primary production and processing

8.3 Farmers and those working with animals are more exposed to a range of zoonotic pathogens (707, 708) including *Campylobacter*, and farm animals are known to be important sources of human *Campylobacter* infections (709). For

example, a number of case-control studies have reported significant positive associations between contact with farm animals, and campylobacteriosis (318, 710). Similar observations have been reported in relation to poultry and meat processing workers (709, 711, 712)

8.4 A recent study of stakeholder perceptions, attitudes and practices which reduce zoonotic risks in the human food chain, indicates that farmers, along with many other groups involved in the food chain, have a generally positive attitude towards risk prevention measures (713). However, a number of studies have reported that, for example, biosecurity compliance on poultry farms is significantly influenced by a number of factors including personality traits, experience, education and training (714). Similarly, recognition of the value of prevention measures may not always lead to their implementation (713, 715, 716), despite general agreement that appropriate biosecurity and other interventions can be effective in reducing the prevalence of *Campylobacter* (475). Some studies have suggested a clear inverse relationship between willingness of farmers to adopt biosecurity measures and the estimated associated costs (716, 717).

8.5 The possibility of inadvertent indirect transfer of *Campylobacter* between flocks on boots, clothes, and other contaminated surfaces in biosecurity ante-rooms is well recognised (503, 515). Thus, recent work has highlighted poultry farm workers as a potential primary vector for *Campylobacter* transmission into broiler flocks, and suggested that prevention of direct contact between farm staff and broilers can prevent or postpone the development and dissemination of *Campylobacter* within flocks (718). In current practice, commercial and operational pressures on catching and thinning processes can make it difficult for those involved in catching and thinning of poultry flocks to comply with desirable biosecurity standards (520, 719)

The Catering industry

8.6 Catering businesses continue to be the most common setting for foodborne disease outbreaks (296, 720, 721). For this reason, a number of FSA campaigns have focused on the catering industry (722-724)

8.7 The catering industry faces the same risks and concerns in relation to safe storage, handling and processing of chicken and chicken meat, as exist at other

stages of the human food chain. Thus, it is important that caterers recognise and treat raw chicken as likely to be contaminated with *Campylobacter*. As such, chicken carcasses, meat and offal should be handled in a manner which minimises cross contamination of other foodstuffs, food contact surfaces and utensils (Strachan et al 2011). However, it can be difficult to achieve and maintain effective control of such cross contamination in restaurants and catering outlets, bearing in mind the complexity of the interlocking production lines inherent in their activities. Poor hygiene during poultry meat preparation leading to cross contamination between raw meat and ready to eat food have been frequently reported as the main causes for outbreaks of campylobacteriosis in the catering industry (296, 369, 720, 725-727).

8.8 In some cases, the risks of campylobacteriosis related to the catering industry may be affected by staff age profiles, ethnic/cultural diversity, and the general pattern of relatively rapid turnover of transient, casual and part-time staff. Thus, some of the older generation "have always washed their chicken" while the younger generation have been correctly informed that washing chicken can spread *Campylobacter* across many food contact surfaces. Cultural culinary practices also affect the way different ethnic and regional restaurants prepare and cook food. There is good evidence to show that many ethnic food business operators within the UK serve raw products to match their nationality's food culture (728). In relation to rapid staff turnover, appropriate training to ensure that new/returning staff fit effectively within catering operations can pose challenges to many catering operations (729, 730).

8.9 While the general cross contamination risks posed by contaminated chicken carcasses and other meats in other parts of the human food chain apply equally to catering activities, particular concerns have emerged in relation to the risks associated with the preparation and undercooking of *Campylobacter* contaminated chicken liver within the catering industry (284, 309)

8.10 Raw livers from a range of food animals have been shown to be contaminated frequently with *Campylobacter*, i.e. chicken 81%, pig 79%, sheep 78% and cattle 69% (295, 392, 417, 725, 731). However, livers from these different species are used very differently within the catering industry. Pig, sheep, and cattle livers are much more likely to be thoroughly cooked in dishes such as faggots (traditionally pig

liver), lamb's liver and bacon casserole, haggis and beef liver and onions. That said, there have been some reports of food poisoning associated with consumption of lamb's liver, including some cases involving respected restaurants (732).

8.11 Nevertheless, in overall terms, the risks posed by *Campylobacter* contaminated chicken livers are significantly higher, because they are more likely to be very lightly cooked (i.e. flash fried) and served or made into a parfait or pate. Such mild heat treatment can be insufficient to inactivate *Campylobacter* present in/on these chicken liver products.

Catering and Chicken Livers

8.12 Current food preparation and processing practices in the catering industry correctly place considerable stock, as part of their HACCP due diligence, on sourcing food materials from reputable suppliers as an important means of sourcing food ingredients which are safe food, and do not contain harmful bacterial contamination. Unfortunately, recent studies have shown that most UK chicken livers are contaminated with *Campylobacter* (295, 417). Recent studies have established that *Campylobacter* contaminate the exterior surface and interior tissue of chicken livers. For example, Firlieyanti et al. (733) reported *Campylobacter* on the surface of 87% samples of chicken liver, and 83% in the deeper tissues, reemphasising the importance of cooking chicken livers thoroughly. Such reports suggest that the chance of consuming *Campylobacter* when eating inadequately cooked chicken liver is higher than 80% (433). Inadequately cooked chicken livers should therefore be regarded as an inherently hazardous food (284).

8.13 Many caterers use the "once cook" method where chicken livers are seared and then blended with butter, cream and seasoning before being left to cool, set and served. Recently, a number of celebrity chefs, have advocated that chicken livers not be cooked for too long, and should be left pink in the middle with some "bounce", implying they should not be thoroughly cooked. Some chefs suggest that chicken livers will reach "blushing pink" when cooked for 4 minutes, and others suggest that after two to three minutes of cooking the cores of chicken livers usually reach about 70°C. Such uncertainties led the FSA to highlight the importance of an adequate time/temperature treatment (penetrating into the core of the cooked liver) rather than

colour change, in making chicken liver safe. Similarly, the New Zealand Ministry of Primary Industries have advise that colour is not a reliable indicator of effective cooking to avoid campylobacteriosis, and that caterers in New Zealand should cook liver until it is no longer bloody in the core (734).

8.14 These and other reports have prompted FSA to commission a study specifically to devise a commercial/catering scale treatment to reliably destroy *Campylobacter* in chicken liver (295, 309). This study noted that liver cores did not uniformly achieve 70°C during the above treatments, and that the thickness of the liver slices significantly influenced heating and cooling rates. This report concluded that adequate cooking of the pate at 68°C by a "twice cook" method (searing of livers prior to blending and a second cook in an oven *bain marie*) is sufficient to destroy all *Campylobacter* on and in chicken livers. The *bain marie* cooking method which entails a second water bath cook has been reported as more effective in destroying *Campylobacter* cells, while maintaining a pleasant colour and texture in the final product (720).

8.15 A recent study noted differences between what chefs think consumers want, and current customer expectation in relation to chicken liver "rareness" (296). Photographs of a range of degrees of cooking were used to assess chefs' ability to identify safe cooking of chicken liver. Most chefs correctly identified safely cooked livers, but overestimated the public's preference for rareness, thus tending to serve rarer liver than consumers expect. This study estimated that 19%–52% of livers served commercially in the United Kingdom fail to reach 70°C, and predicted *Campylobacter* survival rates of 48%–98%. The above disconnect between supply and demand may increases the risks of *Campylobacter* infections among consumers. It also confirms the wider need for the effective use of objective methods of accurately determining product time/temperature treatments (e.g. timers/thermometers) in the catering industry, with less reliance on touch or sight to evaluate degrees of "doneness", especially in relation to wider consumption of higher risk "raw/rare" dishes such chicken livers and beef burgers (296)

8.16 The overriding defence against *Campylobacter* in the catering industry is to have a well-documented and practised HACCP system coupled with strong management oversight and leadership to ensure that staff consistently adhere to the

food safety controls essential to effectively control *Campylobacter* and other foodborne pathogens within catering environments (730).

Risks in domestic settings

Overview of risks in domestic settings

8.17 *Campylobacter* has a low infectious dose; a dose of 800 cells have been shown to cause illness (283, 735), This means that foods such as raw chicken entering a domestic kitchen pose significant direct risks if subsequently consumed without being adequately cooked, and significant indirect risks as major sources of cross contamination of other raw/ready to-eat, or previously cooked foods. Relatively minor departures from recommended hygiene and cooking practices can result in infection. Research has shown that *Campylobacter* can spread significant distances within the kitchen and that the bacteria can survive for long periods in such environments. Thus, any attempt to reduce *Campylobacter* infections must address domestic practices as well as the levels of infection and practice at other points in the food supply chain.

8.18 People's attitudes to the risks associated with *Campylobacter* and other zoonotic pathogens within the food chain are influenced by a number of factors including (absence of) appropriate knowledge and inaccurate (optimistic) risk perception (736-738). When people do not follow recommended food handling and storage practices, at retail outlets (739) and/or at home, they are at greater risk of contracting campylobacteriosis and other zoonotic human foodborne illness (740-742). Although there are many gaps in the available data on the burden of foodborne illness associated with domestic food handling and storage practices, some consistent national and international patterns are clear. The majority of sporadic cases of foodborne illness are thought to be associated with food prepared and consumed at home (721, 743-745), and a number of studies have clearly demonstrated the importance of cross contamination within domestic environments as a major cause of foodborne Illness (746-749). For example, the European Food Authority has noted "the household/domestic kitchen" as the most frequently reported setting (38.5%) for food borne illness (721). More specifically, *Campylobacter* can be easily transferred from raw poultry (or contaminated packaging) (750-752), to consumers' hands and kitchen surfaces (433, 741, 753-

756). Thus, within the UK, 80% of *Campylobacter* infections in England and Wales are acquired at home, and *Campylobacter* cross contamination from fresh chicken meat to other foods and hands has been suggested to be the dominant route of human exposure to this organism (757). Such reports have stimulated interest in the development of consumer advice, and a range of interventions, which can be applied in domestic kitchens (722, 758-760). Apart from processing/retail interventions such as “cook in the bag” options, a number of interventions to reduce *Campylobacter* cross contamination within domestic kitchen environments have been developed.

Disinfectant wipes

8.19 Disinfectant/detergent/germicidal wipes, already widely used in hospital and health care environments to limit bacterial persistence and dissemination (761, 762), are now being increasingly used to suppress *Campylobacter* and other significant food borne pathogens in domestic environments, including kitchens.

8.20 Recent studies (763, 764) have established the impact of disinfectant wipes in reducing cross contamination among a number of food related pathogens, including *Campylobacter*, on kitchen surfaces and food handler’s hands. The most recent study of these studies (764) suggested that the use of such wipes on contaminated surfaces reduced the annual risk of *Campylobacter* infections by up to 99.2%. However, a number of studies have indicated that although disinfectant/detergent wipes can reduce bacterial contamination of treated surfaces, the way in which they are used can significantly influence the scale of such reductions, and may transfer (relatively small numbers of) pathogens between surfaces (761, 765, 766). It is therefore important that disinfectant/detergent wipes are used carefully and correctly in domestic (and catering) kitchens.

8.21 It is well established that effective cleaning/decontamination of food contact surfaces requires the contaminating bacteria to be in direct contact with an adequate concentration of bactericidal agent(s) for a sufficiently long period. Traditionally, this is achieved in a two-stage process, i.e. applying and rinsing off a cleaning product (to remove visible dirt, grease, and food debris), followed by treatment with a disinfectant (at the correct bactericidal concentration, for an adequate contact time) (746, 767, 768). Incorrect use of single use disinfectant /detergent/germicide may not consistently achieve adequate reductions in bacterial numbers on food contact

surfaces. Such risks may be more effectively controlled within catering kitchens, working with established HACCP procedures, but the risks may be greater in domestic kitchens, where these wipes are more widely used (760).

8.22 In broader terms, the incorrect use of antibacterial agents, biocides etc, in domestic (and catering) kitchens may increases the overall risks of the selection and spread of antimicrobial resistance (769-775)

Knowledge and beliefs

8.23 Despite the above evidence of the importance of domestic kitchens in cross contamination within domestic environments as a major cause of foodborne Illness (746-749), many consumers continue to believe that the risks of becoming ill from food prepared and consumed at home are low (776, 777) and that food processing plants and restaurants are responsible for most foodborne illness (778, 779). Such optimistic risk assessment may well underlie reported food safety practices among UK, EU, Canadian and US consumers (747, 759, 778, 780, 781).

8.24 Appropriate food safety knowledge is one of the most important requirements in improving food safety practices (778), and numerous interventions worldwide have sought to improve the effectiveness of food safety education interventions directed at consumers (782). Consumer food safety practices are the last line of defence in relation to domestic food safety (742), and can significantly reduce the incidence and impact of *Campylobacter* infections, irrespective of the standards of food safety achieved at earlier stages of the human food chain (783).

8.25 Unfortunately, UK consumer knowledge in relation to the risks posed by *Campylobacter* is lower than their knowledge of other foodborne pathogens. For example, a Food Standards Agency survey in 2014 (784) observed that only 28% of people had heard of *Campylobacter*, compared with 90% who had heard of *E. coli* and *Salmonella*. Actions to improve consumer knowledge and domestic practices in reducing the risks posed by *Campylobacter* form significant components of previous and current FSA activities. For example, the Acting on *Campylobacter* Together (ACT) campaign (724) seeks to bring together the whole food chain to reduce the burden of foodborne illness in the UK, and the FSA Strategy 2015 (785) states that tackling *Campylobacter* in chicken is a priority.

8.26 These activities have contributed to a growing awareness of the importance of controlling the risks associated with domestic exposure to *Campylobacter*, and developing improved methods to both understand and reduce such risks.

New approaches to mapping the 'domestic microbiome'

8.27 In terms of understanding the ecology of *Campylobacter* within domestic kitchens, the last 20 years have seen progress to view the ecology, persistence and dissemination of agents of food related infections within the overall microbial flora in kitchens (the kitchen microbiome)(786, 787). For example, the "Good germs, bad germs: a participatory model for mapping the domestic microbiome" project in which FSA is a research partner, is likely to transform public understanding of domestic hygiene. This type of approach seeks to apply a range of novel systems such as high-throughput sequencing systems (787), culture independent microbial source tracking methods (788) and integrated videography and sampling systems (789). Such systems have the potential to lead to more effective targeted methods of investigating and intervening in the complex microbiome of many food production and processing environments. Thus it is becoming possible to accurately track *Campylobacter* and their related microflora at all stages of the poultry production, processing and preparation (Farm to Fork) chain (790), including commercial and domestic kitchens (786).

8.28 The impact of such systems is likely to be significant. However, recent studies of the microbiology of *Campylobacter* in domestic kitchens reinforce the importance of well recognised and long-established aspects of kitchen hygiene (736, 739, 791, 792), as described in the FSA Kitchen Check, Your Fridge is your Friend, and related campaigns.

Measures to limit infection in the domestic kitchen

8.29 The key measures to control the organism in the domestic kitchen are

- effective cooking (70°C for 2 min)
- prevention of re-contamination after cooking (from people, pets, raw foods, and the environment)

- prevention of cross-contamination during preparation (with contamination coming from washing chicken and spreading *Campylobacter* through splashes, droplets and aerosols, as well as from not washing hands at all or effectively)
- thorough cleaning using detergent, hot water and disinfectant

8.30 In a study of cross-contamination within 15 domestic homes, *Campylobacter* spp. (and other pathogens) were found to be generally distributed over the entire house, with handles, knobs and domestic animals' feet being more contaminated (791). No relationships were reported between the answers to the questionnaires about hygiene practices and the microbiological results obtained.

8.31 A previous report to FSA (490) suggested (recommendation 8.33) that, in light of the fact that basic precautions may not be sufficient to prevent *Campylobacter* cross-contamination, the FSA should consider how best to highlight to consumers the heightened risks associated with foods such as raw poultry. The FSA completed a package of research investigating domestic kitchen practices to inform their thinking about this. In addition to an in-depth qualitative study of kitchen practices in 20 households (793), the Agency's Food and You UK survey reports (794-797) now provide much better and regular quantitative evidence about how adults behave in their kitchens. That survey, set up in 2010, is repeated biennially, and provides quantitative assessments of adults' reported knowledge, attitudes and behaviours in relation to food safety and related matters. Particularly relevant for this report, it asks about people's behaviour in relation to recommended practice for the 4Cs (cleaning, cooking, chilling and cross-contamination). Details of key findings are described in the next section.

8.32 Because barbecuing of raw meats, particularly poultry, may present particular potential risks in terms of cross contamination and undercooking, ACMSF recommended that previous activity by the FSA to provide targeted advice to consumers on improved cooking/hygiene practices when barbecuing should be repeated prior to each summer period (490) (recommendation 8.29). This is now standard Agency practice (798) and other organisations such as the NHS have followed suit (799).

Behaviour in domestic kitchens

8.33 The 2016 Food and You survey (797) found that the majority of people reported behaviours in line with recommended practices, such as always washing their hands before starting to prepare or cook food and after handling raw meat, poultry or fish (86%), storing raw meat and poultry separately from ready-to eat foods and in sealed containers or at the bottom of the fridge (60% of those who stored raw meat and poultry in the fridge). Just under half (46%) said they always used different chopping boards for different types of food (another possible source of cross-contamination). The majority of respondents (81%) reported cooking food until it is steaming hot throughout and 89% reported that they never ate chicken or turkey if the meat was pink or had pink or red juices. For all these recommended practices, the proportions in 2016 were similar to those in 2010, 2012, and 2014.

8.34 Studies of behaviour in domestic kitchens in a wide range of other countries (for example, New Zealand (40, 800, 801), Switzerland (802), Korea (803), Austria (792), Belgium (804) and Canada (779) paint a similar picture of limited adherence to recommended practices, with the exact proportions of people following different practices varying from country to country.

8.35 Food safety week 2015 focussed on four key recommended practices by means of the 'Chicken Challenge' (722) which encouraged people to pledge to 'do our bit to cut *Campylobacter* food poisoning' by adopting four key recommended practices:

- **store raw chicken separately from other food, covered and chilled on bottom shelf of fridge;**
- **don't wash raw chicken because it can splash germs around your kitchen;**
- **wash everything that has touched raw chicken in soap and hot water – your hands and utensils;**
- **check chicken is cooked thoroughly – no pink meat, steaming hot and juices run clear** (784)**.**

Washing poultry

8.36 An previous ACMSF report (490) made a particular recommendation (Recommendation 8.27) that the practice of washing raw meat and poultry be

actively discouraged by the FSA and the food industry. The Agency has since promoted their guidance very actively (805) including Food Safety Week 2014 which focussed on urging the public to stop washing raw chicken, and sought to raise awareness about the risks of spreading *Campylobacter*. This campaign, which included videos, case studies, infographics, Twitter chats, vox pops, press articles and TV coverage, received widespread attention and support, reaching an estimated 32 million people.

8.37 Domestic practices appear to have shown some improvement in this respect. Although a study in 2002 noted that the most people reported washing chicken (805), the proportion of those doing so is decreasing. The FSA Food and You surveys noted more and more respondents reporting that they *never* washed raw meat or chicken, and the percentage of respondents reporting that they never wash raw chicken, increased from 36% in 2014 to 46% in 2016 (797). Within these results, women used to be more likely than men to report *ever* washing raw chicken (59% compared with 49% in 2014). However, this gender difference is shrinking with 39% of women and 41% of men reporting ever washing raw chicken in 2016

8.38 Whilst the practice of washing chicken has declined, it is still carried out "sometimes" by 40% of people surveyed (in 2016, decreased from 54% in 2014) so more work is necessary to further reduce this undesirable process.

The context in which kitchen practices are carried out

8.39 A 2013 in-depth study (793) found that kitchens can be inefficient in terms of design, size and layout; this was particularly so for participants living in social housing and for households with very young children and older adults. Moreover, households used their kitchens for different aspects of domestic life, far beyond food-related activities, and food-related activities were not confined to the kitchen; they also took place in other internal and external spaces within the home. Food-related and non-food related elements of kitchen practice were entangled: household practices incorporated multiple activities, things (such as chopping boards and utensils), people and places in and outside the home flowed together. The cleaning of floors, work surfaces, food and utensils, was often entangled within other elements of kitchen practice rather than being a discrete practice within the households.

8.40 The above study noted that pets were often fully integrated as members of a household, and their care was not necessarily separated from other kitchen practices; it was not uncommon for pets to remain in the kitchen during meal preparation. Domestic pets can carry *Campylobacter* spp. A number of other studies have found similar integration, often accompanied by a lack of knowledge that *Campylobacter* can be transferred from pets to family members in these circumstances (806-808). *Campylobacter* may be transferred to humans during direct contact i.e. stroking dogs, dogs licking people's faces, etc. or indirectly, by wider cross-contamination of the kitchen environment, food contact surfaces etc. The relative significance of each of these different routes is as yet unclear (412).

What influences domestic kitchen practices?

8.41 The 2013 in-depth study (793) found that households' logic and principles often related to 'rules of thumb' about 'how things are done'; such principles were inconsistently drawn on by study households, particularly in relation to washing meat, poultry and fish; and salad and vegetables. Participants did not see 'Expert' knowledge as being better than knowledge based on experience.

8.42 In the UK, the Food and You survey (797) found that common sources of information about food safety practices cited by respondents were family and friends (47%), product packaging (41%) and the internet (28%). The proportion using these three sources was higher than in 2012 and 2014. A third (30%) said they used food TV shows or cooking programmes, this was similar to Waves 2 and 3. Nineteen per cent of people said they did not look for information on food safety practices (21% of men and 17% of women) and older people were most likely to say they did not look for this type of information.

Variation in risk and behavior by socio-demographic factors

8.43 In the UK, the Food and You Survey (796) has found consistent patterns of difference by age and gender in domestic kitchen practices. Women are generally more likely than men to report food safety practices in line with recommended practice, for example, always washing their hands after handling raw meat (89% reported doing so in 2016 compared with 83% of men) and always cooking food until it is steaming hot throughout (85% compared with 76% of men).

8.44 Comparing age-groups, the oldest respondents (aged 75 and over) have lower scores on a composite measure of adherence to recommended food safety practices (IRP)[1] and are less likely than those in the middle age groups to report some behaviours in line with recommended practices (hand washing, food storage, and use of use by dates). In particular, respondents aged 75 and over are less likely to report always using different chopping boards for different foods (34%, compared to 20-27% of all other age-groups. Younger men aged 25-34 are also less likely to follow recommended food safety practices (they have an overall IRP score of 63, compared with 65 for all men and 69 for all women).

8.45 Variation in kitchen behaviours was also observed by the type of area in the UK Food and You survey. Respondents living in the most deprived areas (based on the Index of Multiple Deprivation) were less likely to report some practices in line with recommended practice for food safety, compared with those in less deprived areas. For example, respondents in more deprived areas (quintiles one and two) were less likely to report always using a different chopping board for different foods (45%) than those in less deprived areas (54% in quintiles four and five). These results are consistent with a recent literature review covering the US and Europe (809) which found riskier food handling practices among minority and low socio-economic status populations. This review concluded that there is a need to continue to identify unique barriers to safe food handling and to determine if scarcity of resources (i.e., cutting boards, paper towels, disinfectants, soap, and thermometers) is widespread and a function of low socio-economic status. If this is so, this issue needs to be identified and acknowledged through either education and/or public health interventions. Another possibility is that these populations are receiving food that is less safe at the level of the retail outlet or foodservice facility. Research examining the quality and safety of food available at small markets in the food desert environment indicates that small corner markets face unique challenges which may affect the quality and potential safety of perishable food. Similarly, a New Zealand study (810) which reviewed cited literature on consumer practice found that the research indicated the influence of demographic factors (age, gender, level of education, income, work

[1] The Index of Recommended Practice (IRP) provides a composite measure of food hygiene knowledge and behaviours within the home; it includes some of the questions from each of the five domains of food safety: cleanliness, cooking, chilling, avoiding cross-contamination and use by dates.

hours, race, location, and culture) in playing a potential role in determining domestic food safety behaviour.

8.46 However, the impact of socio-economic status on vulnerability to foodborne infections (rather than practices that might lead to foodborne illness) is unclear, and the limited existing evidence points to conflicting results. Higher prevalence of gastro-intestinal (GI) infections is often thought to be associated with more advantaged individuals but a recent systematic review looking at the impact of SES on laboratory-confirmed foodborne illness in developed countries suggests that this relationship is not so clear (811). The review identified 16 studies across four pathogens with mixed results, differing by pathogen. For example, Listeria is more common, and *Campylobacter* is less common, in more disadvantaged populations. It should be noted that a reporting bias by socio-economic status cannot be excluded. Inconsistent results have also been observed among studies that have used syndromic definitions of GI infections, with some reporting higher rates of GI infections among those in lower socioeconomic groups [4, 11, 12] and others observing the opposite [13, 14]. These results clearly demonstrate the differing findings within this area of research. Either way, socio-economic status should be considered when targeting consumer-level public health interventions for foodborne pathogens.

8.47 The Food and You survey (796) finds differences in kitchen practices between England, Wales, Scotland and Northern Ireland but no overall pattern of some countries being more likely than others to follow most recommended practices. Washing raw chicken, for example is less common in Northern Ireland (45% of respondents reported never doing this compared with 35% in England) whilst there were no significant differences between countries in the reported use of different chopping boards.

8.48 Respondents living in London were less likely than those living in other regions to report some behaviours in line with recommended practice, for example, they were less likely to report always using different chopping boards for different foods (34%) along with those in the North East (34%), compared with those in all other regions (50% to 60%). Sixty six percent of respondents living in London reported never washing raw chicken, as did 65% in the West Midlands, compared with lower

proportions in the North East, North West, East, South East and South West (44% to 50%).

Variation in risk by ethnic minority group

8.49 There are also variations in kitchen practices between different ethnic groups, although there is less evidence than for other socio-demographic variations. This is largely because many studies (including the UK Food and You survey) are not sufficiently large to provide reliable comparisons between ethnic subgroups. Special studies have to be carried out, with the US being at the forefront of this work; they are often small-scale, or concentrated in particular geographical areas, so the findings cannot be extrapolated to the ethnic minority populations in general. It is also known that people of minority ethnicity groups in the US experience greater rates of foodborne illness, including salmonellosis and campylobacteriosis.

8.50 The limited body of research concerning food safety knowledge and practices among ethnic minority groups tends to focus on general food safety knowledge and practices and has shown risks similar to or greater than in white ethnic groups. A recent literature review covering the US and Europe (809) found riskier food handling practices among minority and low socio-economic status populations. One of the reviewed studies of Puerto Rican women preparing a "Chicken and Salad" meal at home observed microbial contamination of the meal preparers' hands and of kitchen/utensil surfaces (812). Participants who considered food safety as "very important" were less likely to test positive (for *Staphylococcus aureus*) on hands. Contamination on post-handling chicken, counter/cutting board, and salad was positively associated with contamination on participants' hands.

8.51 Focus groups aiming to identify culturally specific food handling practices found that, for all three groups (African American, Asian, and Hispanic), extended time to transport food from retail to home was common (813). Other culturally unique behaviours within groups included using hot water (Asian, Hispanic) or acidic solutions (African American, Hispanic) to clean raw poultry; purchasing live poultry (Asian, Hispanic); cooking poultry overnight (African American); preparing bite-size pieces of meat prior to cooking (Asian, Hispanic).

8.52 A large telephone survey, using questions modified by these findings, compared food handling practices among 'minority and Caucasian consumers' (the terms used in the report), including the behaviours identified (814). Washing raw poultry was a prevalent behaviour among both minority and Caucasian consumers, but was even more common for ethnic minority groups. In addition, the study found that 'minority consumers' were more likely to

- purchase live poultry
- purchase eggs unrefrigerated
- cook offal
- cook a whole turkey overnight.

8.53 These studies highlight the need to understand food handling practices of people in ethnic minority groups and to develop culturally appropriate safe food handling messages for immigrant and minority sub-groups of a country's population. The evidence outlined from other multiracial/ multicultural countries is sufficient to suggest that there are likely to be 'item and process' factors which increase the risks posed by *Campylobacter* and other zoonotic pathogens in the UK and that some research should be undertaken here to explore this further.

Attitudes to and influences from food poisoning

8.54 Overall, the evidence shows that the public have a low risk perception towards food poisoning and a limited understanding of *Campylobacter* and its potentially dangerous health impacts. People see food poisoning as mild or uncommon – with previous experience and optimism bias making it hard for them to imagine serious harm, or recognise the need to change their practices (758, 815). They also find it difficult to accept that their own practices may be risky, and assume that "bad" food poisoning happens to other people/out of home.

8.55 The 2010 FSA Forum report on *Campylobacter* (816) noted that there were low levels of awareness about *Campylobacter* among consumers specifically, although consumers did have an acute awareness of the potential health risks related to chicken if it wasn't stored, prepared and cooked properly. Respondents felt that this awareness had resulted from the many television programmes about chicken in the recent past. However, the most well-known risk was *Salmonella*, with

respondents saying that extensive media coverage of the issue in the past had raised awareness among the public. This low level of awareness of *Campylobacter* was confirmed in the 2016 FSA Forum report on "Consumer acceptability of *Campylobacter* levels in chicken"(758).

8.56 There is a general sense that people have not been affected by *Campylobacter* so therefore their personal kitchen habits are fine. People struggle to picture and keep thinking about invisible risks. Habits, routines, and culture can become embedded practice that is difficult to change – particularly when practice is 'handed down' through culture/family.

8.57 A FSA report on consumer insights (816) found that people become defensive when they feel they are being 'told off' and this can lead people to adhere more strongly to their current food hygiene practices. This report also found that 'Concern for others is much more motivating than concern for self. A sense of duty of care and responsibility is powerful'.

8.58 The 2016 FSA "Food and You" survey summarised respondent views in this area. Around three in four respondents agreed (i.e. 'definitely agree' or 'tend to agree') with statements that they 'were unlikely to get food poisoning from food prepared in their own home' and that 'restaurants and catering establishments should pay more attention to food safety and hygiene' (76% and 78% respectively). Over half of respondents said they agreed that they always avoid throwing food away (62%).

8.59 Forty-two per cent of respondents who had experienced food poisoning reported that they had taken no action as a consequence. The others (58%) had taken some action; the most common being stopping eating at certain food establishments (32% of all those who had experienced food poisoning) and having stopped eating certain foods (17%).

Interventions

8.60 There is not a strong evidence base to the efficacy of any single intervention to improve people's food hygiene practices at home. This reflects the NICE guidelines on Behaviour Change, which did not find strong evidence to support one particular

theoretical framework, or intervention that delivered behaviour change at scale and permanently.

8.61 A Swiss study (802) used the Health Action Process Approach (HAPA) as a theoretical framework; HAPA proposes that engaging in healthy behaviour consists of two processes: forming an intention (motivation phase), followed by a stage of planning to act and action (volition phase). The central finding was that volitional variables (such as self-efficacy and planning) could predict follow-up behaviour, above and beyond previous behaviour, in a sample where active behaviour change was implemented and possible. An Australian study (817) found that providing a cue to action and reminders built food-safety habits that resulted in changes in food safety behaviours. These studies offer directions for future preventive measures and risk communications

8.62 Researchers in the Netherlands used a transdisciplinary approach, involving interaction between both the social and natural sciences, to examine the effect of consumer risk information on human disease risks in the domestic environment (818). They chose to focus on the “disgust” impact of food poisoning and recruited a set of participants who prepared a salad with chicken breast fillet carrying a known amount of tracer bacteria. The amount of tracer that could be recovered from the salad revealed the transfer and survival of *Campylobacter* and was used as a measure of hygiene. This was introduced into an existing risk model on *Campylobacter* to assess the effect of the information intervention both at the level of exposure and at the level of human disease risk. The study showed that the information intervention supported by the emotion "disgust" alone had no measurable effect on the health risk. However, when a behavioural cue was embedded within the instruction for the salad preparation, the risk decreased sharply. The behavioural cue was an additional couple of sentences in the instructions that said ’Take the best possible care when preparing the salad to prevent bacteria being present in the salad. Pay special care to avoid cross-contamination, which occurs when juices from raw meat come into contact with other food, fruit, and utensils.

8.63 Another study (819) evaluated the efficacy of an intervention to increase awareness of food safety during the preparation of raw poultry among first year

college students. Knowledge and self-reported safe poultry preparation behaviour were assessed and, despite the high overall knowledge at the first measurement point, the students had several knowledge gaps, which were successfully overcome by the intervention strategy.

8.64 The FSA study of communication around *Campylobacter* (816) found that communication, as an intervention to change behaviour, had to resonate with the audience (ENGAGEMENT), provide a reason to change (MOTIVATION) and stick with you (LONGEVITY). They also need clarity on what to change (BEHAVIOUR).

8.65 A recent systematic review of safe food handling among consumers (820) reinforces the difficulties in positively modifying food handling behaviour which are just one element in the complex interaction of ingrained everyday kitchen and wider domestic practices. This review notes that most consumer safe food handling behaviour is unconscious, habitual and routine, with individuals tending to have strong confidence in their abilities, and therefore their behaviour is not likely to be changed by increasing knowledge alone. The review notes two types of frameworks that can be used to assist further investigations and interventions: social theories of practice (821) and the theories of planned behaviour, as used in the aforementioned Swiss and Australian studies. It also reviews the increasing evidence that greater progress may be achieved by focusing on those sections of the population who are currently not “set in their ways”(822). Such sectors include children and young adults, as well as others undergoing changes in lifestyle or health status (823-825).

Shopping Behaviour

8.66 A few studies have reported the different aspects of shopping behaviour and the potential of *Campylobacter* food poisoning.

8.67 The 2010 FSA Forum report on *Campylobacter* reported, “Consumers want to spend as little time as possible thinking about what food to buy and how to prepare it. Therefore, they relied on prior shopping habits to help them save time. Brands acted as a signpost for quality and what a consumer could expect from a product and meant that while a consumer may not have purchased this particular product before it would embody the qualities of products they had purchased in the past.

Intra-store brands acted in a similar way and labels such as “finest" or “basics" helped consumers to make similar decisions.”

8.68 A shop-along observational study (739) was conducted in the US to determine actual shopping, transportation, and storage behaviour of consumers who purchase raw poultry products. Neither hand sanitizer nor wipes were observed in 71% of visited grocery store meat sections. Plastic bags were available in the meat sections for 85% of the time, but only 25% of shoppers used the bag for their raw poultry purchases. During checkout, the poultry was bagged separately from other products 71% of the time. A majority of shoppers stored raw poultry in the original package without an additional container or overwrap.

8.69 Another US study (826) investigated the occurrence of total bacteria, coliforms and *Escherichia coli* on handles and seats of shopping trolleys. A total of 85 trolleys (carts) in parking areas of grocery stores in five major metropolitan areas across the US were examined. Coliforms were detected on 72% of the trolleys, with *E. coli* found on 51% of these. The findings emphasise the need for improved sanitation of shopping trolleys and baskets to reduce consumer exposure to pathogens and microbial infections.

Eating out

8.70 Eating out is another potential source of *Campylobacter* food poisoning although, as discussed in section 8, people probably over-estimate the risks from eating out and under-estimate the likelihood of getting food poisoning from food prepared in their own home.

8.71 Eating out is common, has increased over recent decades and may still be increasing. in the 2016 Food and You survey, almost all respondents (96%) reported eating out or buying food to take away, with 43% doing so at least once or twice a week. Younger respondents were more likely to report eating out at least once or twice a week (60% of those aged 16 to 24 and 55% of those aged 25 to 34 compared with 26%–42% of those in the older age groups). Older respondents aged 75 and over and those in households with incomes in the lowest quartile) were more likely to say they never ate out (15% compared with 1%–7% in the other age groups and in other household income quartiles).

8.72 When eating out, some people take precautions such as considering the cleanliness and hygiene of eating establishments and using the Food Hygiene Rating Scheme (FHRS)[2]. In 2016 nearly three-quarters of respondents to the Food and You survey said the cleanliness and hygiene of eating establishments was important to them when making decisions about where to eat out. Women were more likely than men to say that cleanliness and hygiene were important when deciding where to eat (75% compared with 69% of men). Reported awareness of hygiene standards when eating out was lowest among those aged 16-34 (64%), and highest among those aged 25 to 34 (75%) and 65 to 74 (76%).

8.73 When asked specifically about awareness of the FHRS, 83% of respondents in 2016 reported recognising the images compared with 68% in 2014 and 34% in 2012. In 2016 more than half (54%) of respondents said that a 'hygiene rating/score' was one of the factors they had used for assessing the hygiene of establishments when eating out mention was highest among those aged 16 to 24 (73% compared with 21% of those aged 75 and over), and those living in households with children aged under 16 (65% compared with 49% of respondents in adult-only households)

8.74 Poultry liver consumption has been identified as a risk factor for human campylobacteriosis, as has eating raw or rare chicken. The current culinary trend of serving poultry liver 'pink' (meaning that *Campylobacter* will not have been destroyed) may pose a particular risk when people are eating out in restaurants (it is thought that only very small numbers of people prepare/eat pink poultry livers at home).

Recommendations

8.75 Comprehensive and sustained improvements to reduce to risks posed by foodborne pathogens within domestic kitchens will require actions on a number of fronts. These include continuing development and application of advanced methods

[2] Local authority participation in the Food Hygiene Rating Scheme is voluntary in Northern Ireland and England. However, since its launch in October 2010, the scheme has been adopted by all areas of Northern Ireland and all but one local authority in England. The FSA recommends that businesses should display the stickers and certificates at their premises in a place where people can easily see them when they visit. In Wales the scheme is now running in all areas, and display of rating stickers was made mandatory in November 2013. In Scotland, all 32 local authorities have now launched the FHIS, an equivalent scheme.

to refine our knowledge of the ecology of these organisms, as well as sustained progress in improving people's knowledge and practice within such environments.

We recommend that

8.76 the FSA continues to monitor behaviours in the kitchen through the Food and You survey, and uses it to help determine priorities as to which recommended practices require the greatest guidance and campaigning. (Section 7)

8.77 the FSA and industry continue to give priority to reducing the practice of washing chicken (section 7.1)

8.78 the FSA continues to raise awareness amongst consumers and caterers on best practices to avoid cross contamination from raw food to cooked and ready to eat food (through separate utensils/ chopping boards/storage and handwashing).

8.79 the FSA continues to raise public and caterers' (and celebrity chefs') awareness of the risk to human health posed by undercooked chicken livers and provide guidance on effective cooking methods. This is important because since the last ACMSF report (2005), there has been more of a cultural shift when preparing parfait and pâtés reducing to a once lightly cooked method rather than the more traditional frying followed by a *bain marie* second cook which is not as effective in eliminating *Campylobacter.*

8.80 the FSA issues more specific guidance around time/temperature combinations in novel cooking techniques such as sous vide and water bath cooking.

8.81 the FSA considers the development or improvement of food safety training materials that are more specific in tackling risky behaviours in the catering environment including online training.

8.82 the FSA continues to monitor socio-demographic differences in kitchen practices and experience of foodborne illnesses, with a view to considering whether more targeting of particular groups is required. (Section 8)

8.83 the FSA should undertake or encourage research into the ethnic group differences in kitchen practices and experience of foodborne illnesses. Since large-scale research would be expensive, we recommend beginning with some

smaller-scale qualitative research, perhaps with a view to including culturally-specific food-handling practices in the Food and You survey. (Section 9)

8.84 the FSA, in collaboration with other appropriate agencies, should continue to produce and deliver educational campaigns on the principles of food hygiene and safety within schools and colleges.

Chapter 9: How new knowledge influences risk assessment

Introduction

9.1 There has been considerable activity, both within the UK and across the world, over the past 11 years since the second ACMSF report on *Campylobacter* was published (490). The activity ranges from improved understanding of the ecology of *Campylobacter*, to assessment of interventions, to surveillance in animals and humans, including improved understanding of behaviours in domestic kitchens. This has involved the research community, industry, other stakeholders, and consumers, as well as a number of government departments/regulatory bodies. The aim of this chapter is to identify what activity has gone well and what has not in terms of understanding and trying to reduce the levels of human campylobacteriosis in the UK. This will determine what general lessons can be learnt, so that activity in the future to combat *Campylobacter* and other related pathogens can be better targeted and more effective.

Methodology

9.2 It is possible to achieve the above by simply reviewing the literature; however, it would make sense to try and contextualise this in terms of risk assessment, a primary function of ACMSF, and how science and evidence has been used to manage the risk (primary function of FSA). This chapter does not aim to provide advice on how risk management should be done, but simply to understand what has worked and what has not in terms of using science and evidence to inform both risk assessment and management.

9.3 A number of potential frameworks can be used to structure this review. These include either the Codex risk analysis (www.codexalimentarius.org) or Risk Governance frameworks (827). The first is closely related to the current food safety legislation (i.e. (828)) and the latter is really an extension which takes into account more formally social science aspects. These latter aspects are of key importance in reducing human campylobacteriosis because of the requirement to achieve effective risk communication and behaviour change (by FBOs, regulatory authorities and consumers), as well as implementation of interventions based upon evidence from the natural sciences. Figure 9.1 provides a schematic of the risk governance

framework. Although the general direction of process is circular, it should be noted that the world is complex, as *Campylobacter* is not a new organism and work has been done on each step over time. So, in reality, each step is ongoing and effort can be described as “hop on – hop off”. The rest of the chapter bases this framework as a scaffold upon which to place, but more importantly contextualise, the lessons learned. The lessons learned are bulleted and include underlined text.

Figure 9.1 Outline of the Risk Governance Framework (827)

9.4 PRE-ASSESSMENT/FRAMING

Framing – setting up the problem

9.4.1 Science inputs to this process in terms of the evidence that exists: human disease rates, carriage in animal populations, levels of pathogens in food etc. Social science can also be used to inform on the values of the different actors (consumers, industry, regulatory authorities etc) that are involved (827). As such this combined evidence can help risk managers decide which problems need to be tackled and to what extent.

9.4.2 Setting up the problem to be tackled should be informed by:

- natural scientific evidence from surveillance, horizon scanning etc.
- social science assessment of the values, concerns and perceptions of the different stakeholders.

Deciding what approaches need to be taken to investigate the problem

9.4.3 This is predominantly the role of risk managers. However, scientific input is required on the potential scientific approaches that are possible (e.g. quantitative risk assessment, analytical epidemiology, source attribution, how to trial interventions, surveys of consumer and stakeholder views etc.) and their strengths and weaknesses.

- Scientific input is required on assessing the strengths and weaknesses of scientific approaches to address the problem at hand (i.e. human campylobacteriosis). In particular, the methods used need to be robust and, where appropriate, comparable with previous or other ongoing studies. See for example, Chapter 5 Risk in the food chain: Poultry, where it should be noted that (a) the nature of the sample and the method used for the isolation of *Campylobacter* spp. from chicken can significantly influence the detection and enumeration of the organism and (b) where artificial infection studies of poultry should be supported by studies in commercial systems. These points are relevant to the other chapters in this report as well.
- Recent "state of art" developments in methodology/techniques need to be properly considered as they can offer step changes in understanding and

progress. For example, see Chapter 2 *Campylobacter* genetics and genomics, which describes the development of sequence-based typing methods (MLST and wgMLST) for *Campylobacter*.

- Coherent, contemporaneous surveillance/monitoring at relevant points along the food chain (from farm, to poultry processing, to catering industry/domestic kitchens to sick humans) that are sufficient to measure the impacts of policies/interventions need to be identified. It is important that this is considered before policies/interventions are implemented so that their effects can be simulated and whether they can be detected by the monitoring/surveillance that is proposed. Chapter 4 on Source Attribution describes how monitoring and typing of isolates from humans and other reservoirs can be used to evaluate the impact of interventions across a human population. These methods are also very important when assessing changes in trend as seen in Chapter 3.

9.5 APPRAISE THE RISK

9.5.1 There are two parts of risk appraisal. The first is risk assessment which is a technical endeavour that may be qualitative or quantitative and is one of the main roles of ACMSF. The second is concern assessment which requires social science inputs.

Risk assessment

9.5.2 Risk assessment comprises four main stages: hazard identification; hazard characterisation; exposure assessment and risk characterisation as defined by Codex Alimentarius (www.codexalimentarius.org).

Hazard Identification

9.5.3 The involves the identification of the hazard, in this case *Campylobacter*, and food or group of foods that may act as the vehicle of transmission.

9.5.4 Characteristics of the *Campylobacter* organism

- There remains a degree of confusion over the number and specificity of various toxins, due partly to the use of different assays, with some culture filtrates being crude and others pure. There is a need for more consistency in approach. (Chapter 1: *Campylobacter* biology and tools for detection)
- Not all *Campylobacter* are the same
  - The behaviour of *Campylobacter* in chickens is strain dependent (Chapter 5: Risk in the food chain: Poultry. *Campylobacter* in broiler chickens: commensal or pathogen)
  - There are *Campylobacter* multi-locus sequence types that are host associated and others that are generalists (Chapter 2: *Campylobacter* genetics and genomics)
  - Some *Campylobacter* strains are not as sensitive to hostile environments as thought originally (e.g. when attached to chicken show higher levels of heat resistance and capable of long-term survival at chill temperatures) (Chapter 1: *Campylobacter* biology and tools for detection)
  - Antimicrobial resistance of *Campylobacters* can affect treatment options (Chapter 1. Campylobacter biology and tools for detection).

9.5.5 Characteristics of the food and environmental vehicle(s) that transport *Campylobacter.*

- *Campylobacter* can be found in the flesh of broiler chicken and exhibit higher than expected *D* values which may impact on the efficacy of cooking in killing these bacteria. As a result, the relative food poisoning risks of internal and surface located *Campylobacter* need to be determined (Chapter 5: Risk in the food chain: Poultry. *Campylobacter* in broiler chickens: commensal or pathogen)

- There is variation in survival of different strains of *Campylobacter* in water and it is likely that this will be the case for other vehicles (soil, faeces etc.) (Chapter 1: *Campylobacter* biology and tools for detection)
- *Campylobacter* has been found in communities in biofilms, can survive for more than one week, and has increased resistance to disinfection in these structures (Chapter 1: *Campylobacter* biology and tools for detection)
- *Campylobacter* survival is poor at low pH (<3.0) and survive better in moister compared to drier conditions (Chapter 1: *Campylobacter* biology and tools for detection).

Hazard Characterisation

9.5.6 This details the spectrum of disease symptoms, the dose response and vulnerable/susceptible populations.

9.5.7 Lessons learned about the disease and its symptoms

- Poor gut health resulting in acid reflux (e.g. individuals on PPI's) increases susceptibility to *Campylobacter* infection (Chapter 3: Epidemiology of *Campylobacter* in humans)
- There is some evidence of protective immunity from human volunteer studies. However, there is a wide variation in *Campylobacter* strains and so protection may not be exhibited for all. (Chapter 1. *Campylobacter* biology and tools for detection)
- *Campylobacter* can become antibiotic resistant at farm level and during treatment of cases. This can have implications for treating cases who have severe symptoms. (Chapter 1: *Campylobacter* biology and tools for detection)
- Reducing human campylobacteriosis in the human population reduces the incidence of Guillain-Barré syndrome (Baker, Kvalsvig et al. 2012) Chapter 3: Epidemiology of *Campylobacter* in humans).

9.5.8 Lessons learned about the dose response

- The ID50 (dose at which 50% of humans challenged with *Campylobacter* become infected) is a more meaningful concept than infective dose. The ID50

for *Campylobacter* has been reported to be approximately 900 cells (Chapter 3: Epidemiology of *Campylobacter* infection in humans)

9.5.9 Lessons learned about vulnerable/susceptible populations

- The most obvious example is the way that the UK population is ageing and the burden of disease, at least in terms of hospitalisations, is already greatest in the 60+ group. Therefore, it is important to target interventions at this group (Chapter 3: Epidemiology of *Campylobacter* infection in humans).

Exposure Assessment

9.5.10 The exposure assessment aims to determine the path of exposure and the frequency at which it occurs. Humans can be exposed to *Campylobacter* from a number of pathways. Listed below are the pathways and lessons learnt

9.5.11 Chicken

- The volume of fresh chicken sold in the UK is 6 times greater than that of frozen, sliced or cooked (Chapter 5: Risk in the food chain: Poultry)
- There have been several outbreaks of *Campylobacter* associated with chicken liver pâté/parfait since the last ACMSF report. This is associated with chefs' preferences and recipes stipulating light cooking of the livers and the fact that the internal tissues of liver can be *Campylobacter*-positive (Chapters 3 and 8).
- Cross contamination from raw poultry to other foods and invasive *Campylobacter* in poultry flesh are both determinants of human campylobacteriosis
- Those birds that are most heavily contaminated cause the greatest risk (Chapter 5).
- The behaviour of washing raw chicken, seen as a risk factor for human campylobacteriosis because of potential cross-contamination, has improved somewhat but is still quite widespread (Chapter 8).

9.5.12 Environment

- The sources of *Campylobacter* originate from domestic and animal faeces that can be ingested by direct contact or indirectly through drinking water etc.

These are the main environmental sources for the other major gastrointestinal pathogens (e.g. *E. coli* O157, *Salmonella* etc). It is likely that these can be controlled by similar measures (Chapter 7).

8.5.13 Other foods and water

- Raw milk continues to be a source of outbreaks of *Campylobacter* in the UK in regions where it is not banned. Pasteurised milk can be a risk where the pasteurisation process fails and the phosphatase test needs to be carried out to ensure pasteurisation has been applied properly (Chapters 3 and 7).
- Produce can be contaminated on farm (e.g. by faeces or irrigation water) or by cross contamination in the kitchen and as such acts as a vehicle of *Campylobacte*r infection for humans. This, combined with the large amount of uncooked produce consumed, makes it the second highest risk factor for *Campylobacter* infection after poultry (Chapters 3 and 7).
- Private water supplies (PWSs) have been demonstrated to be a risk factor for campylobacteriosis. Effective water treatment methods for both small PWSs and public supplies are available. It is important to ensure that these systems are working properly particularly when there are episodes of heavy rainfall which can lead to greater run-off from agricultural fields (Chapters 3 and 7).
- Pigs predominantly excrete *C. coli* and many of the sequence types are either not (or rarely) found in humans and hence pigs have a very low source attribution (Chapter 4).

9.5.14 Person to person transmission

- Although the concentration of *Campylobacter* in the faeces of human cases can be high, person to person transmission is very low (Chapter 3).

9.5.15 Foreign travel exposure

- A significant number of human *Campylobacter* cases are associated with foreign travel and it is probable that many of these are associated with consumption of food (particularly chicken) eaten abroad (Chapter 3).

Risk Characterisation

9.5.16 This provides the estimation of the adverse effects likely to occur in a given population, and should include a summary of the assumptions and sources of uncertainty.

- Source attribution demonstrates that human clinical isolates are most similar to those from chicken followed by those from ruminants. However, the pathway (e.g. cross contamination as opposed to undercooking or environmental versus foodborne transmission) is not elaborated and other methods such as QMRA and/or case control studies are required to be used in conjunction to fill this gap. (Chapter 4)
- Only a small fraction of *Campylobacter* cases is reported (1 in 9) (Chapter 3).
- It is notable that most people think they are unlikely to get food poisoning from food prepared in their own home (Chapter 8).
- Hospital discharge rates provide a measure of those individuals most affected by the disease and are an alternative metric for measuring the disease trend (Chapter 3).
- DALYs and/or QALYs are metrics that can be used to measure the effect of the disease on the population as well as allow comparison with other diseases (Chapter 3).

Concern Assessment

9.5.17 This provides a systematic analysis of the associations and perceived consequences (benefits and risks) that stakeholders and consumers may associate with a hazard or cause of hazard. The concern assessment ensures that decision makers account for how the risk is viewed when values and emotions come into play.

9.5.18 Consumers

- There remain low levels of awareness and knowledge of *Campylobacter* food poisoning but some consumers want to know more and expect government and industry to act to reduce campylobacteriosis on their behalf.

(http://webarchive.nationalarchives.gov.uk/20180411153611/https://www.food.gov.uk/news-updates/news/2016/15433/consumers-call-for-more-action-on-campylobacter)

9.5.19 Stakeholders

There appears to be limited published social science research examining the views of stakeholders (e.g. food business operators, environmental health officers, supermarkets etc) on their concerns associated with *Campylobacter*. The research that is published has mainly concentrated on farmers. However, there is a well-defined need for their participation (Golz, Rosner et al. 2014) and this has been elaborated in the context of food chain risk analysis (Barker, Bayley et al. 2010). Indeed, several stakeholders have been involved with the FSA *Campylobacter* programme through Acting on *Campylobacter* Together (ACT).

- Independent social science work investigating the awareness, knowledge and motivations of representative stakeholders on *Campylobacter* could provide valuable information for policymakers (Chapter 8).

9.6 TOLERABILITY AND ACCEPTABILITY OF RISK

9.6.1 This step evaluates the risk and is informed by the risk assessment and concern assessment findings (829). There are two main purposes of the evaluation. The first is to carry out a *value*-based judgement of the tolerability and acceptability of the risk. If the risk is tolerable then the benefits (e.g. chicken as a cheap and healthy source of protein or PWS's as a source of drinking water) outweigh the risks. If the risk is acceptable then there will be no requirement to intervene to reduce the risk.

- There is some evidence that consumers want the FSA target levels of *Campylobacter* on chicken to be more ambitious ((http://webarchive.nationalarchives.gov.uk/20180411153611/https://www.food.gov.uk/news-updates/news/2016/15433/consumers-call-for-more-action-on-campylobacter)

- At the same time, there is strong evidence that many consumers continue to believe that the risks of becoming ill from food prepared and consumed at home are low (776, 777) and that food processing plants and restaurants are responsible for most foodborne illness, hence them being slow to change their behaviour to reduce the risks. This suggests that there are concerns about current levels of disease found in the human population, although they may not always be directed at the most relevant targets (Chapter 8).

9.7 MANAGE THE RISK

Formulation of risk management options/interventions

9.7.1 This is primarily the role of the risk manager. A number of potential interventions were investigated as part of the FSA *Campylobacter* research programme and are also being pursued through industry (poultry farms, abattoirs and retailers). Ideas for interventions originate from a range of sources including industry and as a result of scientific research.

- Potential interventions can arise from a number of sources (e.g. industry, scientists, regulatory organisations etc). It is important that when these are listed that the scientific evidence as well as the uncertainties and assumptions associated with it are also collated.

Assessment and evaluation of Risk Management Options

9.7.2 The options can be *assessed* and *evaluated* against a set of consistent criteria. These can include for example efficacy, cost, practicality, minimisation of side effects, public (and industry) acceptability, legality etc. Natural and/or social science methods can be used in the assessment process. For example trialling an intervention that crust freezes chicken can be piloted in a factory and natural science methods can assess its efficacy in reducing the load of *Campylobacter* whilst social science methods (e.g. focus groups, questionnaires etc.) can be used to evaluate this method in terms of public acceptability(557, 830).

- Natural science (efficacy) and social science (practicality, cost, acceptability etc.) methods can be used to assess and evaluate interventions (Chapter 3).

9.7.3 The following are lessons learnt at each point in the food chain where interventions were trialled**.**

9.7.4 *At farm*: a number of biosecurity risks (vertical transmission, crossover between flocks, contaminated water/feed, flies, keeping litter dry, human activity and depopulation) can lead to increased likelihood of flock colonisation/infection and all require “sufficient” control to minimise the chance of this happening. It is unclear, however, what “sufficient” is and will depend on the circumstances of a particular farm.

- Piloting an intervention for one of the above factors can produce meaningless results if confounding factors cause infection of the flock and/or there is insufficient statistical power in the method (Chapter 5).

9.7.5 *In the factory: p*rocessing using aids such as lactic acid, acidified sodium chlorate or trisodium phosphate as well as hot water treatment, crust freezing, long term freezing, cooking and setting processing microbiological criteria, can all reduce *Campylobacter* loads on carcases and subsequently the potential to reduce human disease.

- These processes are not widely implemented for various reasons including legality, practicality, cost etc. Carrying out a multi-criteria analysis of interventions at an early stage will help inform on their suitability (Chapter 5).

9.7.6 *Retail/Supermarket/Catering sector and at home*

- *In the catering sector,* the HACCP system is dependent on well trained, knowledgeable staff (Chapter 7).
- *In the catering sector and at home* effective cooking is important and this is best done by making appropriate use of a thermometer (Chapter 8).
- *At home and in the catering sector* colour is not a reliable indicator of thorough cooking of chicken livers. Objective methods to measure time and temperature using timers and thermometers are required (Chapter 8).

- *At home and in the catering sector*: proper use of biocides/wipes needs to be properly explained – if not used properly can increase the risk (Chapter 7. People's attitudes and behaviours regarding risk)
- *At home and in the catering sector*: cooking trends can affect the safety of foods (e.g. pink liver, complex dishes and fast preparation times) (Chapter 8).
- *At home:* appropriate food safety knowledge is one of the most important requirements in improving food safety practices, and numerous interventions worldwide have sought to improve the effectiveness of food safety education interventions directed at consumers, with some but insufficient success. Continued monitoring of kitchen behaviours should be used to help determine priorities as to which recommended food safety practices require the greatest guidance and campaigning
- *At home: ageing population* Particularly when also factor in that older people are more at risk because they are less likely to follow recommended food safety practices (Chapter 8). More generally, the FSA needs to continue to monitor socio-demographic differences in kitchen practices and experience of foodborne illnesses, to identify whether more targeting of particular groups is required.

Other

9.7.7 There are also some generic lessons that need to be learnt

- When carrying out pilot work to determine the efficacy of an intervention (whether that be application of a process aid such as lactic acid, or introduction of reminders to improve domestic kitchen practices) to reduce *Campylobacter* levels on raw chicken it is important to determine the statistical power of the trial. For example, how many samples need to be tested to have an 80% chance of detecting a 1 log reduction in *Campylobacter* counts (724).
- When using naturally contaminated chicken in an intervention study it should be noted that prevalence and load can be variable. This should be considered at the outset and built into the design of the study (724).
- There has been a poor uptake of rapid methods for detection of *Campylobacter* by the industry. The reasons for this are multifactorial. There

need to be drivers in place for these methods to become commonplace (e.g. checking for *Campylobacter* in the flock prior to harvest) (Chapter 1).

- Control options for internal versus external contamination of chicken flesh with *Campylobacter* will be different at all steps along the food chain except from at farm (Chapter 1).
- Red meats are a low risk for food-borne *Campylobacter* infection and existing controls, particularly blast chilling, are effective. However, source attribution indicates that red meat livestock contribute to human infection but this is most likely due to environmental transmission (Chapter 7).

Selection and Implementation of Risk Management Options

9.7.8 A management decision is required on which option(s) should be chosen. It is likely if this is to be done on farm, at abattoir, retail, or catering then this would need to be decided by industry. This decision should be informed by the relevant natural and social science evidence. The decision may be straight forward if one option dominates others but if this is not the case techniques such as multi-criteria analysis and cost-benefit analysis will be required.

- Interventions can be ranked based on their efficacy (497). However, this is only one factor that needs to be considered when selecting interventions for implementation (Chapter 5).
- The cost of *Campylobacter* infection is expensive GBP 50 million per year (Chapter 3). However, the cost savings in reducing the burden of disease do not pay for the costs of interventions (e.g. carried out by industry).
- Both the natural and social science evidence needs to be assessed when selecting an intervention. It is important that this information is available to the stakeholder(s) who are carrying out the intervention. (Chapter 8)
- There is growing evidence on the effects of *Campylobacter* on bird welfare and crop profitability which are drivers for better *Campylobacter* control on farm (Chapter 5).
- Irradiation and chemical washes have been found to be unacceptable to consumers even when consumers are informed about the food safety risk. (Chapter 5).

Implementation of Risk Management Options

9.7.9 A number of interventions have been put in place at different points along the food chain:

- Many of the interventions that are or can be implemented rely on the voluntary participation of stakeholders and/or consumers. Social science can inform in this area.
    - Reducing the numbers of people washing raw chicken relies on campaigns by FSA/FSS that reach broad sections of the population and explain clearly the counter-intuitive advice that washing is riskier than not washing, along with proximate reminders on packaging carried out by some retailers to inform those preparing the food to change their behaviour
    - campaigns such as “pink chicken” run by FSS relies on those preparing food to change their behaviour and cook chicken thoroughly.

Monitoring of Option Performance

9.7.10 The performance of the option can be monitored in a variety of ways. For example, an intervention on farm to reduce flock prevalence can be monitored; (i) to ensure it is being implemented appropriately on farm; (ii) in terms of monitoring the flock prevalence; (iii) in the prevalence and loads of chickens at abattoir/retail; and (iv) this can be followed through to determine whether there are changes in the disease incidence in the human population. An example where a series of interventions were simultaneously carried out was in New Zealand in primary production, processing, retail and consumer education (148). However, although it was successful in reducing human incidence of the disease, because multiple approaches were tried at once it is difficult to identify which were the most successful.

9.7.11 The reasons for the recent change in trend in *Campylobacter* incidence in the UK in 2016/17 could also be difficult to disentangle for similar reasons.

- Appropriate scientific surveillance/monitoring methods should be in place to determine effectiveness of interventions and ideally measurement of

associated reductions in human disease. These should be decided upon **before** the intervention is implemented so that baseline data can be collected.

- Campaigns aimed at changing people's behaviour also require evaluation. This needs to cover awareness (whether target audience is aware of them), what they were about and whether the behaviours sought were put in place. This can be very complex to establish but that is no reason not to attempt it.

9.8 COMMUNICATION AND PARTICIPATION

9.8.1 This is described by Renn (827) in general terms as follows: "… includes not only informing people of a risk or of a risk management decision, but also establishing the two-way dialogue needed at all stages of the risk handling process – including communication between those responsible for taking risk-related decisions and those responsible for providing the knowledge on which the decisions are based. Excellent communication is particularly important for the involvement of stakeholders in participative risk-related decision making and conflict resolution and for ensuring that they can make informed choices about the risk, balancing factual knowledge about it with their own interests, concerns, beliefs and resources."

9.8.2 The terms of reference of ACMSF are "To assess the risk to humans of microorganisms which are used, or occur, in or on food, and to advise the Food Standards Agency on any matters relating to the microbiological safety of food."

9.8.3 Hence ACMSF is a committee that provides knowledge that can be used to inform risk management decisions. The main role of ACMSF is to provide natural science knowledge but inevitably as is already described in this report the risk from *Campylobacter* is also dependent on human actions/behaviours and as such social science knowledge is also very relevant.

- That there are a number of places in the risk governance process which are dependent upon natural and/or social science evidence and it is important that these are communicated to FSA and other relevant stakeholders appropriately. Further, the generation of the scientific knowledge, where appropriate, should involve the active participation of stakeholders and consumers.

Discussion

9.8.4 The risk governance framework has been used to select the main findings of the report together with the main lessons learnt. The framework is a vehicle in which to contextualise and articulate these findings. In particular, it emphasises the roles of the natural and social sciences in assessing the risk of *Campylobacter* and where scientific knowledge can be used to inform management options. It identifies areas where there is a strong knowledge base as well as others where more work may be required.

9.8.5 The framework itself has been helpful in identifying some gaps in initial drafts of the report; however, it has been relatively difficult to ascertain what level of detail is required and, in particular, the challenge of ensuring that the translation of the science is accurate. The framework has the potential to be applied to any risk and so it may also be of value when considering other microbiological risk as well as chemical contaminants and allergens which are also of interest to the FSA.

9.8.6 Whole genome sequencing has the potential to be integrated into the steps of risk assessment. For example, a genome wide association study of E. coli O157 identified single nucleotide polymorphisms correlated with attachment (Pielaatt et al., 2015). Further the genetic profiles of virulence genes have the potential to be correlated with severity of disease. These examples have the potential to be used as part of the hazard identification and hazard characterisation processes. There may also be the potential to use WGS in the exposure assessment process. For example, SNPs that are markers for improved survival through the food chain. These examples indicate that there could be potential for WGS methods to be used in risk assessment.

Recommendations

9.8.7 To investigate the potential of integrating whole genome sequencing into risk assessment.

9.8.8 FSA to review whether the risk governance format, or something similar, is helpful for risk managers in identifying, considering, and evaluating the natural and social science being used to inform policy.

9.8.9 ACMSF to consider the format of the risk governance framework to determine if it is helpful when conducting risk assessments and review of the literature for FSA, in particular, in deciding and considering the relevant natural and social science data that is required as well as in understanding the role of science and how it interfaces with risk management.

Chapter 10: Conclusions and Recommendations

Chapter 1

1.51 Increased understanding of mechanisms of stress response and biology of *Campylobacter* has revealed a number of alternative mechanisms that allow *Campylobacter* to survive under stress conditions but this has yet to lead to development of new strategies for improved control. We recommend that research is undertaken to determine the impact of genetic diversity in *Campylobacter* spp. on the ability of the bacteria to survive in and respond to hostile conditions found in the poultry food chain.

1.52 As the previous ACMSF report concluded, *Campylobacter* spp. are sensitive to low pH and low a_w stress conditions (e.g. desiccation), and commonly used disinfectants, dying off relatively rapidly compared to other foodborne bacterial pathogens. In addition, alternative processing technologies such as irradiation, and high-pressure processing are generally effective in destroying *Campylobacter*.

1.53 Studies investigating tolerance to aerobic conditions that have been published since the last ACMSF *Campylobacter* report indicate that some strains can become hyper aerotolerant, surviving much longer than aero-sensitive strains and there is some suggestion that these may be more virulent than aero-sensitive strains. This suggests that *Campylobacter* spp. may not be as fragile as previously thought.

1.54 There is little evidence of biofilm formation by *Campylobacter* spp. Simple attachment to and survival on surfaces and in existing biofilms of other species are more likely to contribute to *C. jejuni* survival in food-related environments.

1.55 There is some evidence supporting the view that survival of *Campylobacter* may be assisted by other organisms such as *Acanthamoeba* and *Pseudomonas* species. Further work in this area is required to determine the significance of these findings.

1.56 Most studies investigating heat resistance report relatively small *D* values, indicating that *Campylobacter* spp. are relatively heat sensitive compared to other infectious bacteria. There is general agreement that when *Campylobacter* spp. are attached to chicken meat, higher *D* values are reported. A small number of studies

published since the last ACMSF report that used large pieces of poultry immersed in boiling water or fried, indicate unusually long times would be required for complete destruction. No other studies have reported these unusually high heat resistance values. Before considering the impact of the two heat resistance studies reporting unusually high *D* values when cooking chicken meat, and considering changes to cooking instructions for meat processing facilities, catering or cooking in the home, further work should be carried out to determine if these results are reproducible by other workers, and in this further work, it is critical to accurately measure the coldest point in the meat being cooked. Depending on results, further research could be carried out to establish the mechanisms under-lying the markedly increased heat resistant of *Campylobacter* cells attached to surfaces and particularly on chicken skin and muscle.

1.57 It is recommended that the public health significance of the VBNC state is explored further.

1.58 It is becoming increasingly possible to assess antimicrobial resistance in *Campylobacter*, as in other bacteria, wholly from whole genome sequence (WGS) data. We therefore recommend that determination of AMR from WGSs becomes accepted as standard for *Campylobacter* (See also Chapter 2).

1.59 Methods for recovery of *Campylobacter* are now well established, with standardised reference methods available for detection of the bacteria from foods. These methods will usually be those required to be used by legislation or specifications and will ensure better data comparability between laboratories, production sites and countries. Developments in molecular approaches allow rapid characterisation of different *Campylobacter* species and MALDI-ToF has also been successfully applied for this purpose.

Chapter 2

2.41 At the time of writing (summer 2017), nucleotide sequence analyses had enabled substantial advances to be made in the biology of *C. jejuni* and *C. coli* over the preceding twenty years. Robust methodologies had been established, which enabled: (i) precise isolate characterisation; (ii) high-resolution outbreak investigations; (iii) the establishment of the population structure of *C. jejuni* and

C. coli; (iv) investigations into *Campylobacter* evolution; and (v) improved understanding of the pathways of human infection though attribution analyses. In the immediate future, improved and even more cost-effective means of conducting these analyses can be anticipated, although it is likely that the most dramatic reductions of cost occurred in the 2000-2017. The development of high resolution near-patient characterisation, preferably from complex clinical specimens, remained a major goal which could be anticipated to be achieved in near future. Other than perhaps resolving multiple infections, this technology is unlikely to transform understanding of human infection. A technological development that has the potential for a major improvement in understanding, to be discussed elsewhere in this report, is the development of improved attribution methods on whole genome sequence data. Finally, in line with other foodborne pathogens (263), it is likely that cgMLST methods will become the international standard method for *Campylobacter* typing.

Recommendations

2.42 That sequence-based typing remains the basis for the characterisation of *Campylobacter jejuni* and *Campylobacter coli*.

2.43 Where practicable, sequence-based typing is best achieved using WGS data and the cgMLST analysis approach. When WGS is not practicable or achievable, a combination of conventional MLST and single locus typing (*porA* and *fla* typing) approaches can be used.

2.44 Regard should be given to the possible impact of developments in:

- **(i) Nucleotide sequencing technologies that enable near patient and complex sample analysis;**
- **(ii) Improved attribution to source using WGS data.**

Chapter 3

3.51 We recommend that the Food Standards Agency and its equivalents in the devolved administrations continue to work closely with their counterpart Health

Protection organisations to maintain routine surveillance for gastrointestinal pathogens in general and *Campylobacter* in particular.

3.52 We recommend that the Food Standards Agency and its equivalents in the devolved administration continue to monitor *Campylobacter* levels on chicken carcases at retail sale.

3.53 We recommend that the FSA should continue to warn the public of the health dangers of raw (unpasteurised) milk.

Chapter 4

Risk assessment

4.38 The valuable contribution made by genetic source attribution to estimating the relative contributions of different sources to human disease could be enhanced by (i) validating and optimising accuracy from existing methods and data; (ii) applying reporting standards explicitly report validation results and present adjustments or sensitivity analyses, to include the impact of inaccuracies or uncertainties identified by validation.

4.39 Establishing well sampled and validated datasets, with the appropriate metadata within the larger less structured genetic data databases are essential to provide reference data for source attribution. Planning and initiating collection of these data is needed to ensure that this resource will be in place when needed and to maintain longitudinal data to support analyses that consider change over time and provide intelligence to guide risk management.

Research

4.40 Developing and testing source attribution methods that utilise informative data across the whole genome as the cost of these data falls and availability increases is needed to optimise and identify the limits to these data and this approach.

4.41 Genomic attribution should be integrated with other approaches to maximise its value. This includes: (i) combining source attribution analysis with epidemiology and risk assessment; (ii) use in integrated *Campylobacter*

surveillance across animals, food and humans; and (iii) sampling studies to support this work.

Chapter 5

5.30 We recommend that there is a need to better understand:

- **The population diversity of *Campylobacter* spp., principally *C. jejuni*, in terms of infection biology in chickens and impact on gut and general health, welfare and performance.**
- **The genetic mechanisms used by *C. jejuni*, in particular, to damage gut mucosa and spread from the intestine to edible tissues.**
- **Innate immune responses of different chicken types to different *C. jejuni* strains to inform vaccine development**
- **Innate immune responses of different chicken types to inform the selective breeding of more *Campylobacter*-resistant chickens.**
- **The role of gut microbiota in either preventing or facilitating the colonisation of that organ by *Campylobacter* spp.**
- **Where ever possible and practical, chicken infection studies should be done at a scale that is relevant to industry practice.**

5.76 **It is recommended that the practice of introducing hygiene barriers is adopted throughout the industry to reduce the number of flocks colonised by *Campylobacter* spp. Further measures to reduce the risk of contamination of flocks by contaminated catching equipment and catchers need to be explored.**

5.109 The reduction in *Campylobacter* spp. after packaging and during storage could usefully be studied at a more fundamental level to elucidate the inherent factors contributing to these effects which could then be potentially enhanced through the process. **It is recommended that fundamental research is encouraged in this aspect of *Campylobacter* physiology.**

Conclusion and recommendations

5.117 No single practical intervention has been shown to be capable of eliminating *Campylobacter* spp. or even reducing it to acceptable levels in the bird or during processing. Evidence, however, does show that levels can be reduced by a

combination of farm and processing controls that include implementation of improved biosecurity measures on farm e.g. hygiene barriers in sheds, time-controlled depopulation and in the process e.g. optimisation of existing processing, application of thermal processing (hot or cold). This has been shown to be capable of reducing contamination significantly in recent years from a position where over 30% of chickens on retail sale in the UK had >1000log$_{10}$ cfu per g of *Campylobacter* spp. on the skin to <7%. This has required significant investment in resource and capital but further progress will need to be made to ensure the burden presented by chicken to consumers reduces further.

Farming

5.118 ***It is recommended that the farming industry continues to implement high measures of biosecurity incorporating all of the elements to reduce opportunities for the introduction of* Campylobacter *spp. into the shed. In addition to the recognised controls of litter, water, feed, animals, human activity, flies, etc. the following areas are potentially of significant additional benefit and should receive further consideration for adoption/evaluation by the industry: litter moisture control; time managed thinning; hygiene barriers; antimicrobial factors (microbiome and bacteriophage).***

Processing

5.119 *A number of processing techniques have been demonstrated to achieve significant reductions in* Campylobacter *spp. One of the most important elements in reducing contamination in the processing plant is optimisation of current processing equipment to minimise spread of contamination e.g. plucking and to reduce contamination e.g. inside outside washing. Other technologies that have shown promise and where the industry is recommended to continue adoption and further investigation to enhance efficacy include thermal processing (water and steam) and rapid surface chilling.* ***It is recommended that the factors leading to reduction in* Campylobacter *spp. during the shelf life of the product should be elucidated as this may provide opportunities for additional controls.***

Consumer

5.120 *The continued presence of* Campylobacter *spp. on chicken necessitates the ongoing education of the consumer in cooking and cross contamination controls.* ***It is recommended that the FSA continues to highlight these controls to consumers and industry provides clear labelling advice on storing, preparing, handling and cooking of chicken.***

Collaboration

5.121 ***A key factor in the initial success achieved by the industry in reducing the levels of* Campylobacter *spp. in UK chicken was a full supply chain approach and the importance of promoting an open, collaborative approach is recommended for this and other industry challenges****.*

5.122 ***Much of the improvement in farm and processing measures to reduce the colonisation and contamination with* Campylobacter *spp. has been undertaken in the large poultry processing sector and it is recommended that the FSA, industry assurance and sector bodies ensure that all farms and processors involved in the production of chicken are encouraged to adopt similar standards.***

Chapter 7

7.1.14 Red meat presents a low risk for food-borne transmission of pathogenic *Campylobacter* spp. to consumers.

7.1.15 Available evidence indicates that existing process controls, especially chilling of carcasses, provide an effective means for control of *Campylobacter* along red meat supply chains.

7.1.16 The high prevalence of *Campylobacter*, including *C. jejuni*, among red meat livestock on farms combined with existing attribution data indicates that environmental, non-food borne, pathways for human infection likely exist.

Recommendations

7.1.17 Regular structured surveillance for *Campylobacter* contamination of red meat at retail, updated at least every 5 years, would enable on going assessment of changes in this route for human exposure. Such surveillance is justified by

widespread carriage of *Campylobacter* among red meat species, the potential for contamination during processing and current reliance on the effectiveness of chilling as a critical control point in reducing final exposure *via* retail fresh red meat.

7.1.18 If processing methods were to change in ways that lead to higher contamination rates and levels then this would be concerning since there might be impact on consumer contamination. Therefore, risk assessment steps for future adaptations to red meat processing methods should routinely take account of Campylobacter.

7.1.19 Further research to understand and manage environmental pathways for human exposure linked to primary production of red meat livestock species is justified.

Chapter 8

8.75 Comprehensive and sustained improvements to reduce to risks posed by foodborne pathogens within domestic kitchens will require actions on a number of fronts. These include continuing development and application of advanced methods to refine our knowledge of the ecology of these organisms, as well as sustained progress in improving people's knowledge and practice within such environments.

We recommend that

8.85 the FSA continues to monitor behaviours in the kitchen through the Food and You survey, and uses it to help determine priorities as to which recommended practices require the greatest guidance and campaigning. (Section 7)

8.86 the FSA and industry continue to give priority to reducing the practice of washing chicken (section 7.1)

8.87 the FSA continues to raise awareness amongst consumers and caterers on best practices to avoid cross contamination from raw food to cooked and ready to eat food (through separate utensils/ chopping boards/storage and handwashing).

8.88 the FSA continues to raise public & caterers (and celebrity chefs) awareness of the risk to human health posed by undercooked chicken livers and provide guidance on effective cooking methods. This is important because since the last ACMSF report (2005), there has been more of a cultural shift when preparing parfait and pates reducing to a once lightly cooked method rather than the more traditional frying followed by a bain marie second cook which is not as effective in eliminating *Campylobacter.*

8.89 the FSA issues more specific guidance around time/temperature combinations in novel cooking techniques such as sous vide and water bath cooking.

8.90 The FSA considers the development or improvement of food safety training materials that are more specific in tackling risky behaviours in the catering environment including online training.

8.91 the FSA continues to monitor socio-demographic differences in kitchen practices and experience of foodborne illnesses, with a view to considering whether more targeting of particular groups is required. (Section 8)

8.92 the FSA should undertake or encourage research into the ethic group differences in kitchen practices and experience of foodborne illnesses. Since large-scale research would be expensive, we recommend beginning with some smaller-scale qualitative research, perhaps with a view to including culturally-specific food-handling practices in the Food and You survey. (Section 9)

8.93 the FSA, in collaboration with other appropriate agencies, should continue to produce and deliver educational campaigns on the principles of food hygiene and safety within schools and colleges.

Chapter 9

Recommendations

9.8.6 FSA to review whether this format, or something similar, is helpful for risk managers in identifying, considering, and evaluating the natural and social science being used to inform policy.

9.8.7 ACMSF to consider the format of the attached to determine if it is helpful when conducting risk assessments and review of the literature for FSA. In particular, in deciding and considering the relevant natural and social science data that is required as well as in understanding the role of science and how it interfaces with risk management.

Annex A

ACMSF *Ad Hoc* Group on *Campylobacter*

Terms of Reference

To assess the actions that have taken place since the publication of the Second *Campylobacter* Report and make proposals to advise the FSA in evolving its strategy for reducing the incidence and risk of foodborne *Campylobacter* infection in humans.

Outputs

- The subgroup will evaluate the outcomes to date and prepare a report to advise the FSA in its evolving strategy for reducing foodborne illness and in its statutory functions.
- The subgroup will present its report to the main ACMSF Committee for endorsement

Membership

Professor Sarah O'Brien (Chair)
Mrs Joy Dobbs
Professor Tom Humphrey
Mr Alec Kyriakides
Professor Martin C. J. Maiden
Professor Noel McCarthy
Professor Peter McClure
Professor David McDowell
Mr David Nuttall
Professor Norval Strachan
Dr Dan Tucker
Mrs Ann Williams

Secretariat

Dr Manisha Upadhyay (Scientific Secretary)
Mr Adam Hardgrave (FSA policy representative)
Mr Adekunle Adeoye
Ms Sarah Butler

Annex B

Source Attribution Methods

The Dutch Model

The Dutch model (831) is the simplest way to estimate the attribution of a particular genotype (e.g. ST) to a source, when the frequency distribution of each type is known for each source. If p_{ij} represents the frequency of type *i* (e.g. ST 19) in source *j* (e.g. poultry) then the attribution score of type *i* in source *j* is given by

$$Score_{ij} = \frac{p_{ij}}{\sum_{j} p_{ij}}$$

where the summation by *j* considers all the sources where data exist (e.g. cattle, sheep, wild birds, chicken, turkey etc.).

At single locus ST level this model does not guarantee that all STs will generate an attribution score that will enable them to be attributed to each source. This is because human types that are not found in the animal reservoir cannot be attributed. However, if the microbial sub-typing data produces genetic information exists at multiple loci, then the Dutch Model can make use of the frequency of each individual allele at each individual locus, and estimate attribution even for STs that are not present in the animal reservoirs. In particular, at allele level the frequencies $p_{a_{ijk}}$ can be calculated for each allele a_{ijk} of all isolates from the animal reservoirs. Where i is subtype, j source and k the loci index and n the number of loci.

The attribution score of bacterial subtype *i* in source *j* is

$$Score_{ij} = \frac{\prod_{k=1}^{n} p_{a_{ijk}}}{\sum_{j}\left(\prod_{k=1}^{n} p_{a_{ijk}}\right)}$$

where $p_{a_{ijk}} = BetaInv(0.5, 0+1, N_{isolates}+1)$ if its frequency is zero (*BetaInv* fn in Excel). This assumes that we have no prior knowledge of $p_{a_{ijk}}$ and so is maximally noncommittal or conservative.

Population STRUCTURE

This is a Bayesian clustering model designed to infer population STRUCTURE and to attribute individuals to population groups (Pritchard, Stephens et al. 2000). The program has been used successfully for 7 locus Campylobacter MLST genotyping data (145). Each isolate is attributed on the basis of a training dataset consisting of isolates from known populations (i.e. set USEPOPINFO to 1). The algorithm calculates the frequency of each particular sequence type in each population. Based on these frequencies, the probability of an isolate (e.g. a human isolate) belonging to a population group (e.g. source includes fish, bovine, ovine, poultry, swine etc.) is calculated. This is generally repeated 10,000 times using the Markov chain Monte Carlo process with 1,000 burn-in steps.

Hald Model and Modified Hald Model

This model was developed in Denmark for the attribution of human salmonellosis (410). This "Danish *Salmonella* source attribution" model uses a Bayesian framework with Markov Chain Monte Carlo simulation to attribute sporadic laboratory-confirmed human *Salmonella* infections caused by different *Salmonella* subtypes as a function of the prevalence of these subtypes in animal and food sources and the amount of each food source consumed. The model takes into account the uncertainty for all these factors and also includes travel as a possible risk factor.

This model was improved by (141) to include the introduction of uncertainty in the estimates of source prevalence and an improved strategy for identifiability and is called the "Modified Hald Model". This modified Hald does not include information on amount of food consumed as is the case for the Dutch model.

In summary, the modified Hald model achieves source attribution by comparing the frequencies of human infections caused by different pathogenic subtypes (e.g. serotypes for *Salmonella* (141)), with the subtype frequencies found in the different sources accounting for potential subtype- and source-dependent characteristics, that may influence their chance to cause human illness (410)).

The model utilises a Bayesian approach to estimate and quantify the uncertainty of the parameters.

Briefly,

$$o_i \sim Poisson\left(\sum_j \lambda_{ij}\right)$$

where o_i is the observed number of human infections caused by subtype i that is assumed to be generated by a Poisson probability distribution, whose mean parameter λ is given by the summation over sources of individual λ_{ij}, which are the Poisson parameters for each subtype i in source j and are given by

$$\lambda_{ij} \sim p_{ij} \times q_i \times a_j,$$

where p_{ij} is the prevalence of subtype i in source j, q_i is the subtype-dependent factor, which putatively accounts for differences in survivability, virulence and pathogenicity for subtype i, and a_j is the source-dependent factor, which putatively accounts for the ability of source j to act as a vehicle of listeriosis.

The attribution score to each source j is calculated as follows

$$Score_j = \frac{\sum_{i=1}^{i=I} \lambda_{ij}}{\frac{\sum_{i=1}^{i=I} \lambda_{ij}}{\sum_{j=1}^{j=N} \sum_{i=1}^{i=I} \lambda_{ij}}},$$

where I is the number of subtypes and N the number of sources.

According to Mullner et al. (141) the following default priors were used for the above-mentioned factors.

(a) Source dependent factor

$$a_j \sim dexp(0.002)$$

(b) Genotype dependent factor

$$log(q_i) \sim Normal(0, \tau),$$

where τ is given by a fairly diffuse $Gamma(0.01,0.01)$ distribution.

(c) Prevalence

The priors for the prevalence (p_{ij}) were chosen to be independent beta distributions,

$$p_{ij} \sim dbeta(\alpha_{ij}, \beta_{ij}),$$

where the parameters α_{ij} and β_{ij} were determined form the posterior distributions of a separate Bayesian analysis of the prevalence data, for each source j and subtype i (141, 831) (see prevalence sub-model below).

Posterior distributions of the attribution proportions $(Prop_j)$ in each source j were obtained by a Markov Chain Monte Carlo simulation implemented in WinBUGS1.4 (http://www.mrc-bsu.cam.ac.uk/software/bugs/). Five independent Markov chains were run, each using 30,000 iterations (10,000 burn-ins). This was sufficient to provide convergence using the method developed by Gelman and Rubin (832).

Applicability to level of molecular analysis: This model is only implemented at ST level.

Asymmetric Island Model

This source attribution model incorporates a Bayesian approach and uses the allelic profile of the sequence subtypes to reconstruct the genealogical history of the isolates (139). The host populations are considered to exist on separate "islands" (e.g. the sheep island). Mutations and recombination occur on each island. Migrations between each reservoir (island) into the human population are used to estimate the degree of attribution to each source. This model has previously been applied to *Campylobacter* 7 locus MLST data from England (139), Scotland (142) and New Zealand (141).

The Asymmetric Island model assigns each human case to the potential source populations on the basis of DNA sequence similarity. By comparing human isolates to a panel of reference sequences of known source (e.g. cattle, sheep, chickens, pigs, wild birds and turkey), each human case can be assigned a probability of originating in each source population (i.e. an attribution score). The source attribution probabilities are calculated using a statistical model of the way the DNA sequences evolve in the populations of bacteria. In the statistical model, there are parameters representing the processes of mutation, DNA exchange between bacteria (recombination or horizontal gene transfer) and zoonotic transmission between populations. These processes lead to differences in gene frequencies between the source populations, facilitating source attribution. This model also uses a MCMC process which is usually conducted for 100,000 iterations, with the output file written once every 50 iterations. A symmetric Dirichlet (1) prior is used on the proportion of human isolates attributed to sources, in which all sources are considered equally likely a priori (139).

Glossary of terms and abbreviations

AFLP	Amplified fragment length polymorphism
APEC	Avian Pathogenic *E. Coli*
a_w	Water activity: A measure of the availability of water for the growth and metabolism of microorganisms
DNA	Deoxyribonucleic acid, the genetic material of humans, bacteria, some viruses, etc. It is a polymer of nucleotides connected by sugars
Genotyping	Distinguishing and grouping organisms by their content of genetic information.
Microaerophilic	Relating to an organism that needs oxygen, but less than that present in air.
Microbiota	The population of microbes living within, e.g. the intestine.
Thermophilic	Thermophilic campylobacters are those which grow well at 42°C and 37°C, but not at 25°C.
PFGE	Pulse field gel electrophoresis
Phenotyping	Distinguishing and grouping organisms by their appearance and/or physiological (functional) properties
RAPD	Random amplification of polymorphic DNA
Recombination	The process of creating new combinations of genes with characteristics different from those in either parent
Ribotyping	Characterising bacterial isolates according to their ribosomal RNA pattern.
RpoS	RNA polymerase, sigma S

CspA,	the major *cold-shock protein* in *Escherichia coli*
RpoH,	The heat-shock sigma factor
Rifampicin	An antibiotic used to treat some bacterial infections
Coccoid cells	Spherical (or near-spherical) bacterial cells
Biofilm	A film of micro-organisms
Periplasmic	Present in the space between the inner and outer membrane of gram-negative bacteria
Antigenic	Stimulating an immune response
Coliforms	Gram-negative bacteria present in the digestive tract of animals and humans
z-value	The increase in temperature (^{o}C) required for a 10-fold decrease in the *D*-value
cfu/g	Colony-forming unit per gram
superoxide dismutase	An enzyme that alternately catalyzes the dismutation of the superoxide radical into either ordinary molecular oxygen or hydrogen peroxide
MLST	Multilocus sequence typing
Whole Genome Sequencing	A laboratory process that determines the whole DNA sequence of an organism's genome at a single time
SNPs	Single nucleotide polymorphisms. The most common type of genetic variation among people
auto-agglutination	The spontaneous clumping together of red blood cells
Abiotic	Non-living
MPa	Megapascal pressure unit
MPN	Most Probable Number
ISO	International Standardization Organization

Prospective cohort study	A study that follows a group of individuals to see how exposure to certain risk factors affect outcomes over time
Proton pump inhibitors	Type of drug used to reduce acid production by the stomach
Prodrome	An early sign of disease
Ophthalmoplegia	Weakness or paralysis of the eye muscles
Ataxia	A lack of muscle control or coordination
Areflexia	Absence of neuromuscular reflexes.
Gut microbiota	The population of microbes living in the intestine
BIGSdb	Bacterial Isolate Genome Sequence database
Campylobacteriosis	Infection in animals or humans caused by bacteria of the genus *Campylobacter*
Sequelae	Conditions which follow the occurrence of a disease, e.g. late complications or long-term or permanent ill effects
Commensal	An organism (e.g. a bacterium) living in a symbiotic relationship with another in which one species derives benefits while the other is unharmed
Lumen	The interior space of a vessel, e.g. the intestine
Siderophore	Molecules which transport iron across cell membranes
Cytokines	Cell-signalling molecules associated with inflammation and infection
Ratite	A group of large, flightless birds
CCPs	Critical Control Points
SES	Socio-economic Status
FSS	Food Standards Scotland
Zoonotic pathogen	An organism able to cause disease/illness in an animal that is transmissible to humans.

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