Campylobacteriosis is one of the most important bacterial food borne illnesses in humans worldwide. In the last decade of the former century, the incidence of campylobacteriosis was increasing. The increase in incidence has stabilized now but unexplained yearly variation is noticed. One of the important sources for Campylobacter infections in humans is the handling and consumption of poultry meat. An estimated 20-30% is related to poultry meat but 50-80% may be attributed to poultry, suggesting that besides poultry meat, other transmission routes to humans of poultry derived Campylobacter are important. These routes, however, are largely unknown.

Control of Campylobacter in meat producing poultry will reduce the human burden of illness and control of the Campylobacter colonization in broilers (primary production) would be the best approach. Broilers can easily become colonized with Campylobacter and, although risk factors for flocks to become colonized with Campylobacter have been identified, applicable preventive measures in primary production are limited. Implementation of biosecurity measures will contribute but the effect is hard to quantify. Implementing fly-screens, reduction of slaughter age and discontinue thinning are other measures that will reduce the number of colonized flocks at slaughter age. Within the processing stage options that will reduce the risk for humans are: increasing slaughter hygiene, and physical or chemical approaches like irradiation, the use of UV-light, cooking of the meat, freezing carcasses and decontamination of carcasses by chemicals or hot water. Currently, a promising control strategy is to keep colonized and non-colonized flocks separated in the slaughterhouse. This approach creates a production chain with virtually Campylobacter free meat, supplying the fresh poultry meat market. Meat from Campylobacter positive flocks is processed to reduce the Campylobacter concentration on the meat, prior to consumer delivery. This reduced concentration of Campylobacter leads to a reduced exposure of the consumer to Campylobacter and consequently to a reduced burden of illness.

Campylobacteriosis in humans

Campylobacteriosis in humans the most prevalent bacterial gastro-intestinal disease, exceeding salmonellosis in many countries (EFSA/ECDC, 2012) although in the US the incidence of campylobacteriosis is slightly lower than the incidence of salmonellosis (Scallan et al., 2011). Most data are available from industrialized countries. Data from developing countries are scarce and the epidemiology in these countries is different from industrialized countries (Havelaar et al., 2009). The difference may be explained by acquired immunity due to regular exposure. It is clear that immunity plays a role in the susceptibility of individuals and populations for acquiring campylobacteriosis, however, there are still so many knowledge gaps regarding cross-immunity against different types, the level of immunity, that research in this area has high priority in the near future.

In the last decade of the 20th century, the incidence of human campylobacteriosis was increasing exponentially in many countries in the world but the reason for this increase remained unknown (WHO, 2000). The incidence seemed to be stabilized although clear differences per years are observed but largely unexplained. Despite more than 30 years of intensive research it is still hard to control campylobacteriosis in most countries of the world and it is still not clear by what mechanism...
Campylobacter can cause disease in humans and not in any animal species (except for sporadic cases of abortion in cattle and sheep). Campylobacteriosis in humans is characterized by watery or bloody diarrhea, abdominal cramps and nausea (Skirrow and Blaser, 2000). The infection is self-limiting but in a fraction of the patients serious sequelae occur like the Guillain-Barré syndrome, reactive arthritis and the irritable bowel syndrome (Hannu et al., 2002; Hughes and Cornblath 2005, Haagsma et al., 2010).

The role of protective immunity in humans is not well documented but it is most likely that immunity may lead to temporarily protection against re-infection or disease especially in people who are frequently exposed to Campylobacter, e.g. through professional contact.

Sources for human campylobacteriosis

The estimation of sources for human campylobacteriosis (source attribution) is an important tool to identify the most effective intervention strategies for prevention. Source attribution can be done by epidemiological and microbiological approaches. Epidemiological studies have identified the consumption and handling of poultry meat, and direct contact with animals as most important sources for human campylobacteriosis (Friedman et al., 2000; Studal and Andersson, 2000). It is likely that the effect of immunity also confounds the results of case-control studies. This may explain why in rare cases poultry meat is identified as protective factor (Adak et al., 1995; Friedman et al., 2004). Other risk factors for human campylobacteriosis are: direct contact with animals, contaminated drinking water, foreign travel, and the consumption of raw food products. The quantitative attribution of the consumption of contaminated poultry meat was estimated from a Dutch case control study on human gastroenteritis to be 20-40% (Doorduyn et al., 2010). The upper limit of this attribution is derived from a Belgian study on the effect of the withdrawal of chicken meat from the market after an incident with dioxin (Vellinga and Van Look, 2002). In the Netherlands, as a result of the avian influenza outbreak in poultry in 2003, a considerable decrease of sporadic and outbreak-related Campylobacter infections was observed parallel to the reduced consumption of poultry meat (Friesema et al., 2012).

A recent study from the European Food Safety Authority investigated the source attribution of human campylobacteriosis by different methods estimated that handling, preparation and consumption of broiler meat may account for 20% to 30% of human cases of campylobacteriosis, while 50% to 80% may be attributed to the chickens as reservoir (EFSA, 2011).

Campylobacter: microbiological and molecular aspects

Campylobacteriosis in humans is mainly caused by Campylobacter jejuni and to lesser extent to Campylobacter coli. Other Campylobacter species are also reported causing disease in humans but their importance differs in geographical regions. The reported number of the non-jejuni/coli infections is worldwide a small fraction of all Campylobacter infections, therefore this paper focus on C. jejuni and C. coli.

C. jejuni and C. coli are Gram negative bacteria sensitive to many external physical conditions like low water activity, heat, UV and salt. They are much more fragile than Salmonella or Gram positive bacteria like Enterococcus. In contrast to Salmonella, Campylobacter will not multiply on meat samples in the absence of micro-aerobic conditions and non permissive temperatures. The highest concentration of Campylobacter can be found on meat directly after processing (due to cross-contamination from the gut contents to the meat). In all following steps (transport to retail and to consumer kitchen, storage) the concentration may stabilize but is more likely to decrease due to die off of bacteria. The intestines of animals (mammals and poultry) are the amplification vessel for Campylobacter. In the environment Campylobacter can survive when it is protected from dryness but it will not multiply. In slurries and in dirty water Campylobacter can survive up to 3 months (Nicholson et al., 2005). Manure from animals may contaminate surface water with Campylobacter.

Typing of Campylobacter to trace transmission routes

For several food borne pathogens like Salmonella, serotyping and phage typing of isolates are important tools to trace infections and to perform epidemiological studies. Many typing methods have
been described for Campylobacter, both phenotypic (serological and phage typing) and molecular based (review by Wassenaar and Newell, 2000). It has to be noticed that for Campylobacter most typing methods can only be used for tracing infections restricted in time and geographical area. Although further typing of strains is often requested for epidemiological reasons, the biology of Campylobacter (natural competent species with DNA-uptake and frequent rearrangements of the genome) hampers the use of many molecular typing methods to investigate Campylobacter epidemiology. In the recent years Multi Locus Sequence Typing (MLST) has been introduced as a powerful molecular method with a high reproducibility and, because of the fact that it is a sequence-based method, an easy data-exchange between laboratories (Müllner et al., 2010). MLST has been used successfully to trace contamination routes and to estimate the contribution of different sources to human illness (EFSA, 2010).

**Poultry and Campylobacter**

All types of poultry can become colonized with Campylobacter and also wild birds are frequently colonized (Newell and Wagenaar, 2000; Waldenstrom et al., 2002). In contrast with Salmonella, eggs do not contribute to the human campylobacteriosis problem as Campylobacter is not vertically transmissible (Callicott et al., 2006). As most research is done on the chicken meat production and most data are available from this sector, this paragraph will be restricted to the broiler production.

Day old chicken and older animals can easily become colonized with Campylobacter when they are experimentally inoculated. Even with low dose (10 – 100 bacterial cells), they become colonized and start shedding in about 2-3 days with up to 10^6 campylobacters per gram caecal contents. Under experimental conditions, Campylobacter spread rapidly between animals. The colonization with Campylobacter does not lead to any clinical sign in poultry. An association between animal health and Campylobacter has been described. This may be a direct association (impaired health increases the risk for Campylobacter colonization) or an indirect association (e.g. a lack in biosecurity leads to the introduction of Campylobacter and other infectious agents) (Bull et al., 2008). Sporadic cases of vibrionic hepatitis in poultry have been associated with Campylobacter. The causative role, however, of Campylobacter is not proven (Burch, 2005; Jennings et al., 2011). C. jejuni may cause illness in ostriches (Stephens, 1998).

Broilers are free of Campylobacter at day of hatching, so each cycle of broiler starts with a Campylobacter negative flock. In many studies there is a 1-2 weeks lag phase identified prior to detection of Campylobacter in a flock. Several biological explanations are given to explain this lag phase (e.g. maternal immunity, composition of the gut flora). Mathematical modeling of the spread of Campylobacter within a large flock shows that a colonized flock becomes detectable positive only 7 days after the first animal became colonized (Van Gerwe et al., 2005; Katsma et al., 2006). This has to be taken into account when defining the lag phase. Till 7 days only a small fraction of the flock is shedding Campylobacter and it is not likely that in routine surveillance programs these (few) animals will be detected.

Depending on the country (i.e. continent and climate zone), the incidence of positive flocks may vary and there is a strong seasonality in the colonization rate. The dynamics of the seasonality is also strongly dependent on the country. The northern European countries have clearly sharper peaks compared to the more southern countries (Nylen et al., 2002)

Most of the flocks are colonized with multiple strains (Jacobs-Reitsma et al., 1995). This may lead to even more variety in Campylobacter strains as strains may exchange DNA leading to chimera strains and therewith increasing the diversity (De Boer et al., 2001). Broilers may become colonized with C. jejuni and with C. coli. However, at about 6 weeks the majority of strains isolated from broilers is C. jejuni. In older animals, e.g. in organic production, there is a shift towards C. coli (El-Shibiny et al., 2005).

**Risk factors and sources of Campylobacter for poultry**

To be able to implement targeted intervention strategies, the sources and transmission routes of Campylobacter into broiler flocks need to be identified (Newell and Fearnly, 2003). In many different studies several common risk factors were identified: contamination of flocks increases with the age of the animals, with the number of houses on a farm, and the presence of other animals on the farm or direct surroundings (Van de Giessen et al., 1996; Bouwknecht et al.; 2004, Kapperud et al., 1993). In a Dutch study on 10 broiler flocks that were screened for the presence of Campylobacter for 10 subsequent cycles, the risk for a flock to
become positive increased when a former cycle was positive (Katsma et al., 2007). A systematic review based on UK data was published on the risk factors, comprising 159 research papers (Adkin et al., 2005). Partial depopulation (thinning) and multiple house-on-farm were identified as contributing factors associated with increased risk, and hygiene barrier, parent company, and certain seasons of rearing were associated with decreasing risk. The common theme of many of these risk factors is biosecurity.

**Intervention strategies to prevent the introduction of Campylobacter into a flock**

The control of Campylobacter along the food chain would have the highest impact when colonization of animals on-farm can be prevented. Reducing the prevalence of colonization with Campylobacter in the primary production phase will decrease the introduction of high numbers of Campylobacter into the slaughterhouse (Nauta et al., 2007). This may finally result into a low concentration or absence of Campylobacter on the final product. Recent publications about the attribution of poultry-associated Campylobacter strains to human campylobacteriosis through other transmission routes than poultry meat, supports the importance of on-farm control. The identification of risk factors for the introduction of Campylobacter, enables the implementation of specific intervention strategies (Newell et al., 2011). Based upon the risk factors identified before, the possibilities and effect of biosecurity, including the use of fly screens and thinning will be discussed. Additionally, strategies that need more research before implementation into practice (competitive exclusion, bacteriocins, and vaccination) are shortly discussed.

**Biosecurity and thinning**

Theoretically, a high level of biosecurity on farm level should be protective against Campylobacter. Some association (Van de Giessen et al., 1998) is found but even an extremely high level of biosecurity does not guarantee a Campylobacter free flock at the time of slaughter (Jacobs-Reitsma, pers. comm.). Instruction for the farmers regarding increased hygiene may lead to a lower prevalence of Campylobacter as is shown in Nordic countries. Campylobacters are continuously present around broiler houses and even if facilities for biosecurity (anterooms, disinfection for boots, separate clothing and utensils) are present, they must be used consistently in order to prevent colonization of flocks. So apart from technical aspects there is also behavioral aspect involved.

A particular breach of the biosecurity is thinning. This is the process of partial depopulation of broiler houses in order to give more space to the remaining birds for ethical and economical reasons. The practical approach of it may vary from country to country and this may lead to the different opinions whether thinning is a risk factor or not. Crucial is the time interval between thinning and final depopulation of the flock. Differences of this interval in different countries and flock size may account for the opposite opinions whether thinning is a risk factor. There is a common sense that through thinning there is an increased risk of introducing Campylobacter into a flock by machinery and people not taking into account all hygienic measures. Mathematical modeling shows that within one week after infection and start of spreading, the prevalence of colonized broilers in flocks (size 30,000) is low (<1%) (Katsma et al., 2007). Even when up to 100 broilers become colonized at thinning, the prevalence of colonized broilers remains <10% after one week. When final depopulation of a flock takes place one week after thinning, Campylobacter may not be detected in the common surveillance systems. When the interval increases, the prevalence of positive animals in a flock increases and therewith the chance that a flock is found positive.

**Fly-screens**

In the past and in recent years, research is done on the role of flies and the transmission of Campylobacter. Flies can be carrier of Campylobacter and the fly-traffic in and out broiler houses is huge, so flies are identified as a clear risk factor and intervention showed a delayed and reduced campylobacter colonization in flocks (Shane et al., 1983; Hald et al., 2004; Hald et al., 2007). This approach is very promising.

**Competitive exclusion, bacteriocins and vaccination**

**Competitive exclusion (CE)** has shown to be successful in Salmonella control programs in poultry. Several studies on CE in the control of Campylobacter have been published but the effects of CE for Campylobacter are variable. At the moment there is not yet a commercial product available that claims effect against Campylobacter.

A recent development is the use of a bacteriocin added to feed to control C. jejuni in chickens (Svetoch and Stern, 2010). This approach claims to
be effective in the prevention of the colonization but it is not yet commercially available.

There are no commercially available vaccines against Campylobacter in poultry. The development of these vaccines is hampered by three main problems: the antigenic variety of strains, the lack of knowledge of antigens inducing a protective immune response, and the response of the chicken immune system against Campylobacter as commensal organism (De Zoete et al., 2007).

Strategies to eliminate Campylobacter from flocks

Once colonization is established in a flock, close to 100% of the animals become colonized and shed high numbers of Campylobacter (>10⁶ CFU/gram feces), with only a slight decrease of time. Two approaches have been described to reduce the level of colonization. The first one is the use of lytic phages that specifically attach to and lyse Campylobacter cells (phage therapy). Under experimental settings this approach has shown to give a 2-3 log reduction of the Campylobacter shedding (Wagenaar et al., 2005; Carrillo et al., 2005). Risk assessment models predict a significant reduction of risk for the consumer with the 2-3 log reduction on caecal level (Havelaar et al., 2007).

Bacteriophages are present where Campylobacter is present, also on poultry meat, and the phages are safe for public health, public acceptance of using a virus to control Campylobacter, needs attention (Atterbury et al., 2003).

A second approach may be the already mentioned use of bacteriocins (Svetoch and Stern, 2010).

Contamination during transport from farm to slaughterhouse

Several studies have shown that transport crates are the source for negative chicken to become contaminated during transport from farm to slaughterhouse (Hansson et al., 2005). However, this will only give an external contamination of the animals and not a significant colonization in the gut. It has to be noticed that contaminated crates may be a risk for remaining animals after thinning (see before).

Preventing cross contamination in the slaughterhouse

Due to the high concentration of Campylobacter in the intestines, in particular the caeca, chicken carcasses become contaminated at the surface during processing. Carcasses from Campylobacter negative broilers can become contaminated through the machinery when they are processed after a positive flock. However, this contamination results into a lower concentration of bacteria at the surface compared to carcasses from colonized chickens. This contamination has a negligible impact on the risk for humans compared to products from Campylobacter positive flocks (Rosenquist et al., 2003).

Scheduled slaughter and control of Campylobacter

As cross-contamination in the slaughterhouse can hardly be prevented, an effective approach may be to separate positive and negative flocks followed by decontamination of the meat from positive flocks. Theoretically this approach will work but practically it is quite complicated to separate positive and negative flocks (Wagenaar et al., 2006). Knowledge of the Campylobacter status of a flock when it leaves the farm, offers the possibility to direct this flock to a “positive-only” slaughterhouse, whereas negative flocks will be transported and processed in “negative flocks only” slaughterhouses. Testing flocks for the presence of Campylobacter by conventional bacteriological culture techniques needs too much time between sampling and having the results at the day of slaughter. False negative results (Campylobacter could not be isolated from the samples from the farm and the flock is positive at the time of arrival at the slaughterhouse) are reported frequently mainly due to flocks that became positive between sampling and slaughter (Hofshagen and Bruheim, 2004). An option could be to test with PCR at arrival of the slaughterhouse or shortly before they left the farm when samples can be shipped to a laboratory (Lund et al., 2003).

Conclusions

The practical and economical available options to control Campylobacter in the poultry meat production chain are still limited. As the epidemiology
differs strongly from country to country, the effect of control options will most probably differ in different countries.

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