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Campylobacter in primary animal production and control strategies to reduce the burden of human campylobacteriosis

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Summary

Campylobacteriosis is one of the most important bacterial food-borne illnesses in humans. One significant source of infection is the handling and consumption of poultry meat, although other sources also contribute considerably. Controlling *Campylobacter* in broilers reduces the human burden of illness.

Broilers can easily become colonised with *Campylobacter* and preventive measures in primary production have a limited and unpredictable effect. Vaccination, competitive exclusion, bacteriophage therapy and the use of bacteriocins are not yet commercially available. However, measures in the slaughterhouse can reduce contamination in the final product. At present, the most promising control strategy is to keep colonised and non-colonised flocks separate during slaughter ('scheduled processing'). The virtually *Campylobacter*-free meat can supply the fresh poultry meat market, while the meat from infected flocks can be treated to reduce the *Campylobacter* concentration. Meat from infected flocks can be treated by freezing but chemical decontamination appears to be more cost effective. A variant of this scenario is to treat only highly contaminated meat.

The authors conclude that, until new techniques become commercially available, scheduled processing is the most cost-effective approach. Finally, the authors describe trends in antimicrobial resistance in *Campylobacter*.

Keywords

Antimicrobial resistance – Campylobacter – Campylobacteriosis – Food safety – Intervention strategy – Poultry – Poultry meat – Public health – Scheduled processing – Slaughter – Zoonoses.

Introduction

Campylobacteriosis and *Campylobacter*

Campylobacter species are identified as a major cause of bacterial gastroenteritis in humans worldwide (4). Depending on the country, either *Campylobacter* or *Salmonella* is the most frequently isolated bacterial pathogen from cases of diarrhoea (58). During the last decade of the 20th Century, the incidence of human campylobacteriosis increased exponentially in many countries but the reason for this remains unknown (69).

Campylobacteriosis in humans is characterised by watery or bloody diarrhoea, abdominal cramps and nausea (54). The infection is self-limiting but, in a fraction of the

patients, serious sequelae occur, such as Guillain-Barré syndrome and reactive arthritis (26, 32).

In the Netherlands, which has a population of 16 million, it is estimated that, each year, 80,000 cases of *Campylobacter* gastroenteritis result in 18,000 patients visiting their general practitioner, 600 hospitalisations and approximately 30 deaths, mainly among the elderly. The economic costs of campylobacteriosis in the Netherlands are estimated at 21 million euros per year (42), while the yearly disease burden is estimated at 1,200 disability-adjusted life years. In the Netherlands, this figure is comparable to those for tuberculosis and bacterial meningitis (28).

Campylobacteriosis in humans is principally caused by *Campylobacter jejuni* and, to a lesser extent, *C. coli*. Other *Campylobacter* species are also reported to cause disease in humans but their importance differs from geographical region to geographical region. The reported number of non-*C. jejuni* or non-*C. coli* infections is only a small fraction of all *Campylobacter* infections worldwide; thus this paper focuses on *C. jejuni* and *C. coli*.

Campylobacter jejuni and *C. coli* are Gram-negative bacteria which are sensitive to many external physical conditions, including:

– dessication
– heat
– ultra-violet (UV) radiation
– salt.

Campylobacter are much more fragile than *Salmonella* or Gram-positive bacteria, like *Enterococcus*. In contrast to *Salmonella*, *Campylobacter* does not multiply on meat samples in the absence of micro-aerobic conditions.

The highest concentration of *Campylobacter* is found on meat directly after processing. In all subsequent steps in the food chain (for example, transportation to retail, refrigerator storage), the concentration may stabilise but is more likely to decrease, due to die-off of the bacteria.

Campylobacter and animal diseases

Both *C. jejuni* and *C. coli* have a high incidence in companion and production animals, where both species are a commensal gut inhabitant. *Campylobacter jejuni* can cause sporadic cases of abortion in cattle but is not of great economic importance. Sporadic cases of vibrionic hepatitis in poultry have been described, supposedly caused by *Campylobacter*. However, this causative role is suggested only (10); there is little evidence. *Campylobacter jejuni* may cause illness in ostriches (55) and has been associated with diarrhoea in dogs. However, it is not clear whether this species really causes disease in canines or just shows an increased shedding in watery stools, secondary to other causes of diarrhoea. No animal diseases caused by *C. coli* have been described in production or companion animals.

Campylobacter in the environment

Warm-blooded animals are the amplification vessel and thus reservoir for *Campylobacter*. *Campylobacter* does not replicate in surface water, due to the absence of a micro-aerobic atmosphere, the low temperature and lack of nutrients, but it can survive when it is protected from dryness (one of the major threats for *Campylobacter*). Most surface water sources are contaminated by animal manure containing *Campylobacter*. In slurries and in dirty water, *Campylobacter* can survive for up to three months (48).

Sources of human campylobacteriosis

Epidemiological and exposure assessment studies have identified the consumption and handling of poultry meat, and direct contact with animals, as the most important sources of human campylobacteriosis (21, 57). The role of protective immunity in humans is not well documented but it is most likely that immunity may lead to temporary protection against re-infection or disease, especially in people who are frequently exposed to *Campylobacter*, for example, through their work. It is likely that this immunity also confounds the results of case-control studies. This may explain why, in rare cases, poultry meat is identified as a protective factor (2, 22). Other risk factors for human campylobacteriosis include:

– direct contact with animals
– contaminated drinking water
– foreign travel
– the consumption of raw food products, including milk.

Quantitative attribution to the different sources is difficult to establish. From a Dutch case-control study on human gastroenteritis, it was estimated that 20% to 40% of campylobacteriosis is attributable to the consumption of contaminated poultry meat (W. Van Pelt, personal communication). The upper limit of this attribution is derived from a Belgian study. During the dioxin crisis of 1999, sales of chicken meat were prohibited for a four-week period. Over this period, the incidence of campylobacteriosis was 40% lower than expected, based on previous years, but returned to the normal level after the ban on chicken sales was lifted (65). In the Netherlands, as a result of the avian influenza outbreak in poultry in 2003, a considerable decrease in human *Campylobacter* infections was observed. This decrease seemed to correlate to the reduced consumption of poultry (64).

Campylobacter and techniques to trace infection pathways

For several food-borne pathogens, such as *Salmonella*, serotyping and phage typing of isolates are important tools to trace infections and perform epidemiological studies. Many typing methods have been described for *Campylobacter*, both phenotypic (serotyping and phagetyping) and molecular based (68). Genotyping is more commonly used than phenotyping, due to the advantages of molecular methods (which are fast, less labour-intensive and fewer strains are untypable, in comparison to serotyping), and the limited availability of antisera. When investigating outbreaks, any typing method is suitable.

It should be noted that the current typing methods for *Campylobacter* can only be used for tracing infections that are restricted in time and geographical area. Although further typing of strains is often requested for epidemiological reasons, the biology of *Campylobacter* (a natural competent species with DNA-uptake and frequent rearrangements of the genome) hampers the use of large-scale typing. In the past, large-scale typing studies did not result in clear epidemiological conclusions about specific sources. New developments in typing may overcome these old problems. As an example, multi-locus sequence typing (MLST), which has been introduced relatively recently, is a molecular method that is highly reproducible. This method provides results that can easily be exchanged between laboratories and stored in one central database. Preliminary MLST data show that there is a certain correlation between the presence of a specific allele in *C. coli* associated with strains isolated from cattle and the strong association of specific *C. jejuni* sequence types and cattle (19, 44).

In addition, origin-specific markers for strains of *C. jejuni* were suggested by Champion *et al.* (11). If these findings continue to prove true, after further typing of isolates from different continents and sources, both MLST and specific markers could become useful tools for approximate attributions of human illness to different sources.

Poultry and *Campylobacter*

All types of poultry (broilers, layers, turkeys, ducks, fowl, quail, ostriches) can become colonised with *Campylobacter*. Wild birds are also frequently colonised (46, 67). In contrast with *Salmonella*, eggs do not contribute to the human campylobacteriosis problem as *Campylobacter* is not vertically transmissible (20). The most important vehicle of transmission of *Campylobacter* to humans is poultry meat that becomes contaminated during processing. Although turkey meat may also be a source of human campylobacteriosis (7), most research has been conducted on chicken production. Since most data come from this area, this section will deal with *Campylobacter* in broilers.

Day-old chicks and older animals can easily become colonised with *Campylobacter* when they are experimentally infected. Even with low doses (10 to 100 bacterial cells), the birds become colonised and start shedding in about two to three days. The colonisation reaches > 10^6 colony-forming units (CFU) per gram of caecal contents. Infections spread rapidly between animals under experimental conditions. Colonisation with *Campylobacter* does not lead to any clinical signs in poultry.

Broilers are free of *Campylobacter* on the day of hatching, so each cycle of broilers starts with a flock which tests negative for *Campylobacter* infection. In many studies, there is a delay of one to two weeks (the 'lag phase') before *Campylobacter* is detected in a flock. Several biological explanations have been given for this lag phase. However, mathematical modelling of the spread of infection within a large flock shows that, even without factors delaying the spread, an infected flock can only be detected as positive seven days after the introduction of the infection (63, 37). Until seven days have passed, only a small fraction of the flock sheds *Campylobacter* and it is not likely that these few animals will be detected in routine surveillance programmes.

The incidence in positive flocks may vary, depending on the country (i.e. continent and climate zone), and there is strong seasonality in the infection rate. The dynamics of the seasonality are also strongly dependent on the country. Northern European countries have much sharper peaks of incidence compared to more southern countries (49).

Most flocks are infected with multiple strains (34). These mixed infections may lead to even more variety in *Campylobacter* strains as they can exchange DNA, leading to chimera strains and increasing diversity (13). Broilers may become colonised with *C. jejuni* and with *C. coli*. However, at about six weeks, the species most commonly isolated from broilers is *C. jejuni*. In older animals, for example, in organic production, there is a shift towards *C. coli* (15).

Monitoring programmes

Monitoring programmes are implemented to identify trends in *Campylobacter* infections and evaluate the feasibility of control programmes. They can also aid in linking the poultry data to human *Campylobacter* data, to assess the contribution of poultry to the human burden of illness. A good example is the obligatory monitoring of *Campylobacter* in broilers in the European Union (EU), as required by Directive 2003/99/EC (18). This directive

implemented the monitoring of broiler flocks from 1 January 2005, with the European Food Safety Authority (EFSA) as the agency responsible for compiling and reporting data collected by the EU Member States. At this time (June 2006), the EU is unique in instituting this monitoring programme (http://www.efsa.eu.int/science/monitoring_zoonoses/reports/catindex_en.html).

Risk factors and sources of infection for poultry

Since *Campylobacter* is horizontally transmitted into broiler flocks, primary control measures should be implemented at the farm level. However, before targeted intervention strategies can be implemented, the sources and routes of infection for broiler flocks must be identified (47). In many different studies, several common risk factors were identified for the introduction of *Campylobacter* into a broiler flock. Contamination of flocks increases with the following:

– the age of the animals

– the number of broiler houses on a farm

– the presence of other animals on the farm or in the direct vicinity (8, 35, 61).

In a Dutch study on ten broiler farms that were screened for the presence of *Campylobacter* for ten subsequent cycles, the risk for a flock to become positive increased when a former cycle tested positive (37). Recently, a systematic review on risk factors, based on United Kingdom data, and comprising 159 research papers, was published (3). Depopulation schedules (thinning) and multiple broiler houses on farms were identified as factors associated with increased risk. Disease prevention and hygiene measures, the presence of more than one generation of chicks (i.e. broilers and their parents) and certain seasons of hatching were all associated with decreasing risk.

Strategies to prevent the introduction of *Campylobacter* into a flock

The control of *Campylobacter* along the food chain is most effective when the colonisation of living animals can be prevented. Reducing the prevalence of *Campylobacter* infection in the primary production phase decreases high numbers of *Campylobacter* in the following steps. This may result in a low concentration or absence of *Campylobacter* on the final product.

Identifying risk factors for the introduction of *Campylobacter* means that specific intervention strategies can be implemented. In the following sections, the authors discuss the possibilities and effects of biosecurity, multispecies farming and thinning, as well as competitive exclusion, vaccination and genetic resistance.

Biosecurity

Theoretically, a high level of biosecurity on the farm should protect against *Campylobacter*. Some correlation has been found (62), but even an extremely high level of biosecurity does not guarantee a *Campylobacter*-free flock at the time of slaughter (W.F. Jacobs-Reitsma, personal communication). Educating farmers on improved disease prevention measures and hygiene may lead to a lower prevalence of *Campylobacter*. However, conflicting reports come from two Scandinavian countries: Norway reports a positive effect from its education programme but Iceland has not observed any effect (31, 50).

The effects of improved hygiene are hard to quantify. As part of the *Campylobacter* Risk Management and Assessment project (CARMA, available at: htt://www.rivm.nl/carma/index_eng.html) in the Netherlands, a mathematical model was developed to describe *Campylobacter* dynamics in the primary production phase. Improving biosecurity was evaluated as an intervention strategy and identified as a potentially effective approach. However, this could only be established in theory as there is a lack of knowledge on methods that are effective in practice. No researcher can recommend to a farmer how to improve their biosecurity and indicate a specific percentage of anticipated reduction of *Campylobacter*. As increased biosecurity cannot be broken down into specific control measures, it is not clear what investments are needed. *Campylobacter* strains are continuously present around broiler houses and even if biosecurity measures (such as anterooms, disinfection facilities for boots and separate clothing and utensils for each house/worker) are in place, they must be consistently applied to prevent colonisation. Thus, apart from the technical aspects of disease prevention, there is also a behavioural aspect involved, which has not been studied so far.

Research has been conducted on the role of flies in transmitting *Campylobacter*. Flies can act as vectors for *Campylobacter* and the fly 'traffic' in and out of broiler houses is huge, so flies are a clear risk factor (24, 53). Controlling flies leads to both delayed and reduced *Campylobacter* infection in poultry flocks (25).

Multispecies farming

As multispecies farming is a risk factor for *Campylobacter*, recommending that farmers farm only one species sounds reasonable. However, economic analysis has shown that stopping multispecies farming is not an effective approach. For economic reasons, farmers will then increase their numbers of chickens and broiler houses. Banning other livestock may lead to a reduction in *Campylobacter*

infection but, by increasing the number of broiler houses on the farm, one risk factor will simply be replaced by another, and the net result is estimated to be neutral (37).

Thinning

'Thinning' is the process of partially depopulating broiler houses to give more space to the remaining birds for ethical and economic reasons. Although the definition of thinning is the same, the practical approach may vary from country to country. For this reason, there are different opinions on whether thinning is a risk factor. The period of time between thinning and final depopulation of the flock is crucial. Differences in this interval, as well as varying flock sizes, probably account for the range of opinions on thinning as a risk factor.

There is a common perception that thinning causes an increased risk of introducing *Campylobacter* into a flock through inadequate disinfection of machinery and workers. Mathematical modelling shows that, one week after infection (the start of disease spread), the prevalence of infected broilers in flocks (size 30,000) is low (< 1%) (37). Even when up to 100 broilers become infected at thinning, the prevalence of infected broilers remains at < 10% after one week. If the final depopulation of a flock takes place one week after thinning, the infection still may not be detected by common surveillance systems. However, when the interval between thinning and final depopulation increases, the number of animals testing positive in the flock also increases and so does the chance that the infection will be found. In some cases, however, the increased risk of thinning can be entirely attributed to the increased age of the broilers (52).

Competitive exclusion

Competitive exclusion has been shown to be successful in *Salmonella* control programmes in poultry. Several studies on the use of competitive exclusion to control *Campylobacter* have been published but the results are variable. As yet, there is no commercial product that claims good results against *Campylobacter*.

One recent development is the use of a bacteriocin added to feed to control *C. jejuni* in chickens (56). This approach claims to be effective in preventing colonisation but it is not yet commercially available.

Vaccination

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 which induce a protective immune response

– the requirement to provide protection in the very early life stages of the bird.

Several scientific studies show a (partial) protective effect of the humoral response under experimental conditions. A vaccine against *Campylobacter* in broilers must be effective in the very early life stages of the bird, requiring either protective maternal immunity or an innovative approach to induce protective immunity in the young chick.

Genetic resistance

Differences between genetic lines for susceptibility to *Campylobacter* infection are suggested but the data are limited and the molecular background is not yet clear (9).

Strategies to eliminate *Campylobacter* infections from flocks

Once an infection is established in a flock, close to 100% of the birds become colonised and shed high numbers of *Campylobacter* (> 10^6 CFU per gram of faeces).

Over an extended time period, there is a slight reduction in shedding, suggesting that immunity plays a role. However, in the normal lifespan of a commercially housed chicken, this may be limited to approximately one log reduction of *Campylobacter* concentration in the faeces (unpublished data).

A few approaches are reported to reduce shedding in a well-colonised flock. The first is phage therapy. This method uses lytic phages that specifically attach themselves to and lyse *Campylobacter* cells. Under experimental settings, this approach has been shown to reduce *Campylobacter* shedding by two to three logs (38, 66).

Risk assessment models predict a significant reduction of risk for the consumer, with a two-to-three log reduction in the caecal level of *Campylobacter* in poultry (29). However, since the large-scale practical implementation of this method involves several problems (e.g. its application, resistance), phage therapy is not expected to be commercially available within the next few years. Although bacteriophages already exist wherever *Campylobacter* is present, including in poultry, and these phages are safe for human health, using a virus to control *Campylobacter* may not meet with public acceptance unless it is capably and comprehensively presented (6).

A second approach may be the already mentioned use of bacteriocins. Bacteriocins have been proposed as a curative treatment but the results have not yet been published (56).

Conclusion on interventions to eliminate *Campylobacter* infections in primary production

In conclusion, the only currently available and (partially) effective intervention in primary production is to introduce a higher level of biosecurity. Although some practical steps can be suggested in this direction, the biosecurity approach is yet to be developed into a full programme of specific control measures with a defined quantitative effect. Therefore, the balance between costs and benefits can only be defined theoretically. Since other potential interventions and therapeutic strategies are not yet commercially available, a major decrease of infections on broiler farms is not likely in the short term.

Contamination during transportation from farm to slaughterhouse

Several studies have shown that transport crates, during transportation from the farm to the slaughterhouse, are a source of contamination for poultry which previously tested negative for *Campylobacter* (27). However, crates only cause external contamination of the birds, not significant colonisation in the gut. The few animals that do become colonised will have only very low numbers in the intestines.

It should be noted that the people, machinery and crates introduced into broiler houses to catch and transport the birds may well be the source of contamination for the remaining animals during thinning (see 'Thinning', above).

Preventing cross-contamination in the slaughterhouse

Owing to the high concentration of *Campylobacter* in the intestines, in particular, the caeca, the outside surfaces of chicken carcasses also become contaminated during processing. Carcasses from *Campylobacter*-negative broilers can be contaminated by machinery when they are processed after a positive flock. However, this contamination results in a lower concentration of bacteria at the surface when compared to carcasses from colonised chickens. Thus, such contamination has a negligible effect on the final product (51).

Improving the slaughtering process

It is technically possible to make slight changes to the slaughtering process to prevent and reduce cross-contamination. The most serious constraint is that a high number of birds must be slaughtered per hour and cleaning and disinfecting the machinery between every two carcasses is not possible.

There are two potential approaches in the slaughterhouse:

– to prevent cross-contamination
– to decontaminate meat by chemical or physical means.

One powerful method appears to be the newly developed and patented equipment that forces a small amount of faeces out of the cloaca, through pressure on the abdomen, and subsequently removes the faeces with a pulse of water. The aim is to limit faecal leakage during scalding and defeathering. Mathematical modelling predicts that the number of CFUs on a chicken carcass after cooling would decline by a factor of three to ten. However, this method needs to be validated in practice (29).

For decontamination, five methods may be useful. First, irradiation of the meat virtually eliminates the public health risk. However, the costs of this method are relatively high and there is a good deal of public resistance. The second method may be to treat carcasses with, for example, a lactic acid solution (2.5%). This leads to a decrease in the average count of *Campylobacter* on the carcass after cooling, by one to two log units. A third method is crust-freezing of the carcasses with a stream of cold air. This is effective but relatively costly.

A fourth method has been used in a number of Scandinavian countries (Norway, Iceland, Denmark). Carcasses or parts of carcasses from flocks which test positive for *Campylobacter* infection are frozen for several weeks. According to laboratory experiments, the numbers of *Campylobacter* may be reduced by a factor of between ten and 100. The costs are high but risk assessment models predict that this method will reduce the burden of illness considerably (45, 51).

Finally, the fifth method is to heat treat the meat.

Measures for reducing *Campylobacter* can be applied to all flocks. Alternatively, one can decide to process only those flocks which test positive for *Campylobacter* infection in this way. This approach is called 'scheduled processing'.

Scheduled processing to control *Campylobacter*

An effective approach may be to separate positive and negative flocks, followed by decontaminating the meat from the positive flocks. Theoretically, this approach will

work but, in practical terms, it is quite complicated to separate *Campylobacter*-positive and -negative flocks. Positive flocks can be identified at different points along the production chain.

Knowing the infection status of a flock when it leaves the farm means that *Campylobacter*-positive flocks can be sent to a 'positive-only' slaughterhouse, whereas negative flocks will be transported and processed in slaughterhouses for 'negative flocks only'. Where only one slaughterhouse is available, the negative flocks would be processed first, followed by the positive flocks.

A negative flock that is misidentified as positive will not result in any problem except for a possible economic one. However, a positive flock that is misidentified as negative will be treated as 'safe meat' throughout the process, may cross-contaminate truly negative flocks and will be sold without *Campylobacter* reduction treatment.

Flocks can be tested for the presence of *Campylobacter* by conventional bacteriological culture techniques. However, *Campylobacter* die off easily during transportation from the farm to the laboratory. In addition, isolating *Campylobacter* requires at least two days (as well as the time needed to mail samples). False negative results (i.e. *Campylobacter* could not be isolated from the farm samples but the flock tested positive on arrival at the slaughterhouse) are reported frequently, due mainly to flocks that became positive between sampling and slaughter (30). There are two alternatives: either the farmer can use a rapid on-site test just before the flocks are transported to the slaughterhouse or the flocks can be tested by polymerase chain reaction (PCR) when they arrive at the slaughterhouse. The on-site test is currently under evaluation in the Netherlands, whereas the PCR technique is routinely used in Denmark (39).

The efficacy of scheduled treatment was assessed using the mathematical model (37). The reliability of negative test results, which is crucial in this approach, depends strongly on the length of time between testing and slaughter. The sensitivity and specificity of the test appeared to be of minor importance (37).

Is there an acceptable level of contamination?

Dose-response models used in microbiological risk assessment are based on the 'single-hit' principle, i.e. a single CFU of *Campylobacter* can colonise a host and potentially cause illness, albeit with a relatively low probability (23, 59). If the ingested dose increases, the likelihood that one of the pathogens establishes infection will also increase. This implies that there is no 'safe' level of *Campylobacter* on broiler meat since any level may potentially cause disease in the human population. However, the risk of illness at low exposure levels is considerably less than the risk at higher levels. The concentration of *Campylobacter* on chicken meat, and the fraction of these bacteria that is ultimately ingested, vary over several orders of magnitude. Risk assessment models typically show that most cases of illness are predicted to result from high ingested doses, which occur relatively rarely (45). Thus, reducing average exposure or the probability of peak exposure is expected to result in significant reductions of risk to the consumer. For example, Rosenquist *et al.* (51) predicted that reducing the concentration of *Campylobacter* on broiler meat 100-fold may reduce disease incidence 30-fold. So, from a public health perspective, low numbers of *Campylobacter* on broiler meat may be tolerable, as they are not expected to result in a significant incidence of illness. This is also related to the fact that (in contrast to, for example, *Salmonella*) *Campylobacter* cannot multiply outside a warm-blooded host.

Other sources for human campylobacteriosis and intervention strategies

Food

Poultry meat is known to be one of the most important sources of *Campylobacter* for humans. However, *Campylobacter* colonisation in the gut is described for all production animals. *Campylobacter* is mainly a contamination of the surface of the carcass and bovine, ovine and porcine carcasses can also test positive for *Campylobacter* immediately after slaughter. Storage (cooling down) of the carcasses under dry air conditions results in the death of *Campylobacter* and reduced *Campylobacter* counts after a prolonged time. At retail level, the *Campylobacter* contamination levels of non-poultry meat are clearly less than the levels in poultry. It is to be expected that red meat contributes to human campylobacteriosis to a much lesser degree than poultry, but the quantitative contributions of various meats are unknown.

Preventing *Campylobacter* colonisation in cattle and sheep is unlikely to be achievable as farming under increased biosecurity is impossible, due to the type of husbandry. In pork production, there are practical examples of farms with high biosecurity levels. However, poultry production has demonstrated that it is hard to keep a flock free of *Campylobacter* for six weeks. For the much longer production cycle of fattening pigs, it may be a huge challenge to keep the animals free of *Campylobacter*.

Seafood may be contaminated with *Campylobacter* but it is more likely that this will be *C. lari* instead of *C. jejuni* or *C. coli*. *Campylobacter lari* is also a human pathogen but the incidence in the human population is clearly less than that of *C. jejuni* or *C. coli* infections. The only intervention is heating the product. For raw fish products (such as oysters) there are no specific intervention strategies other than depuration of the water or UV irradiation.

Other raw food items may contribute to human campylobacteriosis. Produce may be contaminated through irrigating systems or the use of contaminated manure (36). As much produce is not heated, even a low concentration may be a risk for humans.

Direct animal contact

Direct contact with production animals may be a source of human campylobacteriosis. People working with these animals on a daily basis, for instance, farmers, may be protected against the disease by acquired immunity. This protection is not very well studied and the level of protection and cross-protection will be one of the most challenging topics in better understanding *Campylobacter* epidemiology over the next few decades. People who are incidentally exposed to *Campylobacter*, e.g. visitors to a petting zoo, may contract campylobacteriosis. As companion animals like dogs and cats are often asymptomatic carriers of *Campylobacter*, these animals may also be a source for human campylobacteriosis. Epidemiological studies describe an increased risk of campylobacteriosis for owners of cats and dogs, and case studies describe the disease in humans, due to direct contact with companion animals.

Intervention strategies are solely based on hygiene. At petting zoos, people should be made aware that they should wash their hands after touching animals. However, children are mainly at risk as they do not recognise the risk. For companion animals, the situation is more complicated and there is no other option than to 'live with it'.

Antimicrobial resistance in *Campylobacter* species

Until recently it was difficult to obtain comparable susceptibility data on *Campylobacter* spp. isolated from food animals at slaughter. The primary reason was that there was no standardised methodology to determine the susceptibility of *Campylobacter* spp. (1). In 2004, the agar dilution method was validated for quantitative susceptibility testing of *Campylobacter* spp. by McDermott *et al.* (41). Acceptable limits for the quality control strain *C. jejuni* ATCC 33560, determined by the broth microdilution test, are provided by the Clinical and Laboratory Standards Institute in its most recent guidance documents (M100-S16) (12). This greatly aids in determining quality controlled minimum inhibitory concentration (MIC) data.

An external quality assurance system was co-ordinated by the Danish Institute for Food and Veterinary Research within the EU Fair project (ARBAO-II, QLRT-2001-01146) (5). In this project, ring trials were organised to harmonise the results of susceptibility tests of *Campylobacter* spp. in European countries. Subsequently, MIC data of harmonised quality were reported from different European countries (Tables I and II).

Resistance percentages vary greatly from country to country, based on differences in policies on antibiotic use. In *C. coli*, resistance levels are higher than in *C. jejuni*. The antibiotics of first choice for treating campylobacteriosis in humans are macrolides and fluoroquinolones. Resistance to erythromycin is commonly present in *C. coli* from pigs and resistance to fluoroquinolones is commonly present in both *C. jejuni* from poultry and *C. coli* from pigs.

Trends in resistance to fluoroquinolones in the primary sector have been reported in the Netherlands (43) (Fig. 1). However, macrolide resistance has decreased substantially in pigs. This is potentially related to the ban of tylosine as a growth promoter in 1999, whereas resistance to ciprofloxacin has shown a slow tendency to increase in pigs after its licensing for these animals in the mid-1990s. The first occurrence of fluoroquinolone resistance in *C. jejuni* in 1988, one year after the introduction of quinolones into the poultry industry in the Netherlands, was described by Endtz *et al.* in 1991 (16). Endtz demonstrated the link between the introduction of fluoroquinolones and the simultaneous increase in ciprofloxacin resistance in *C. jejuni* from poultry and human cases of campylobacteriosis. From the 1990s until 2004, a further increase in ciprofloxacin resistance was observed to a level of approximately 40% in veterinary isolates (33, 43).

More recently, several authors have demonstrated that fluoroquinolones rapidly select for resistant mutations during the treatment of poultry and pigs (14, 33, 40, 60). This phenomenon is the basis for the discussion of the public health risks of fluoroquinolones in poultry. Fluoroquinolones are used to treat, for example, colibacillosis and *Mycoplasma* infections in poultry and, as a side effect, the *Campylobacter* spp. present in the intestines of the bird become resistant. Since poultry is considered one of the major sources for *Campylobacter* infection in humans, using fluoroquinolones in poultry indirectly selects for fluoroquinolone-resistant

Table I
Occurrence of antimicrobial resistance among *Campylobacter coli* isolates from pigs, isolated from faeces at slaughter, in European countries in 2002

Antibiotic	Country, number of isolates and resistance percentages					
	Switzerland	Denmark	United Kingdom	France	Netherlands	Sweden
	251	**92**	**706 [a]**	**317 [b]**	**64**	**100**
Ampicillin	–	0	16	12	13	7
Chloramphenicol	0	0	3	–	0	–
Fluoroquinolones	25	8	10	12	12	16
Erythromycin	21	32	85	65	55	0
Gentamicin	0	0	–	0	0	0
Kanamycin	1	–	2	–	–	–
Nalidixic acid	26	8	17	20	11	18
Neomycin	–	1	–	–	1.6	–
Streptomycin	80	52	–	–	88	–
Tetracycline	9	1	79	83	70	1

a) 1999-2000
b) 2000

Table II
Occurrence of antimicrobial resistance among *Campylobacter jejuni* isolates, isolated from chickens, in European countries in 2002

Antibiotic	Country, number of isolates and resistance percentages						
	Denmark	France	Germany	Netherlands	Norway	Sweden	Switzerland
	53	**43**	**82**	**44**	**161**	**84**	**180**
Ampicillin	8	28	0	23	3	10	7
Chloramphenicol	0	–	–	0	–	–	0
Fluoroquinolones	0	30	9	41	< 1	0	12
Erythromycin	0	7	0	0	1	0	< 1
Gentamicin	0	2	0	0	0	0	0
Kanamycin	–	–	–	–	–	–	–
Nalidixic acid	0	36	9	39	0	0	–
Neomycin	0	–	–	5	–	–	–
Streptomycin	0	–	–	0	–	–	6
Tetracycline	2	67	33	32	0	1	4

Source: (5)

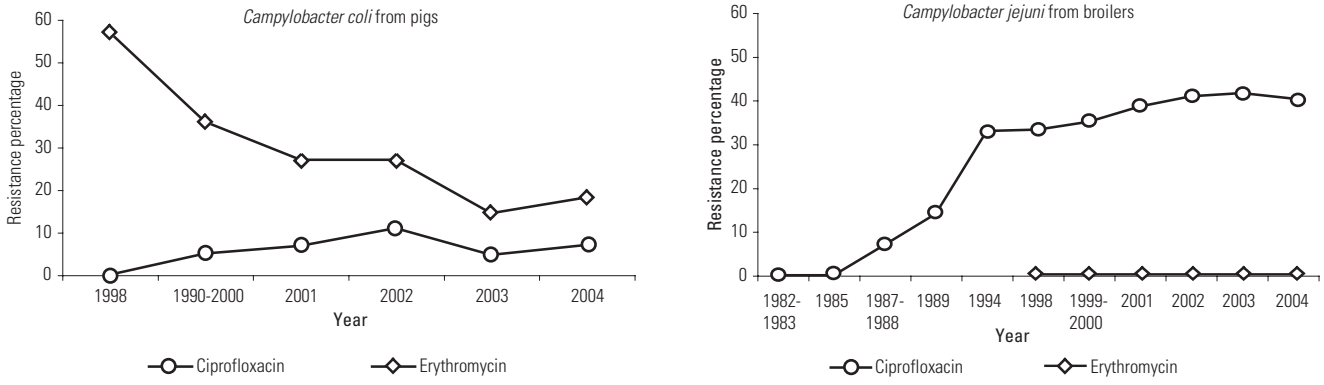

Fig. 1
Trends in resistance to erythromycin and ciprofloxacin in *Campylobacter* spp. isolated from pigs and broilers in the Netherlands from 1998 to 2004 (and since 1982 for ciprofloxacin resistance in *C. jejuni*)

Campylobacter strains in human infection, and may contribute to problems in human therapy.

Similar trends in fluoroquinolone resistance in *Campylobacter* spp. have been described for human clinical isolates by Engberg *et al.*, (17), following the introduction of fluoroquinolones into animal and human treatment. As a result of increased fluoroquinolone resistance in human *Campylobacter* isolates, the United States Food and Drug Administration banned the use of fluoroquinolones in poultry from September 2005 (http://www.fda.gov/oc/antimicrobial/baytril.pdf).

In the EU, from January 2005, a new zoonoses Directive (2003/99) was implemented, prescribing not only the annual monitoring of *Campylobacter* spp. in food animals, but also the annual surveillance of antimicrobial resistance in *Campylobacter* strains isolated from the major food animal species: cattle, pigs and poultry (18). As a result of Directive 2003/99/EC, new Community Reference Laboratories (CRLs) have been designated to monitor antimicrobial resistance (in general) and *Campylobacter*. Specific tasks will be to standardise and control the analytical methods for isolation, species identification and susceptibility testing. An important source of variation in susceptibility test results is the sampling strategy used. The CRL, in co-operation with EFSA, must develop adequate guidance documents so that existing control programmes in the Member States can be successfully harmonised.

■

La présence de *Campylobacter* dans les élevages et les stratégies de prophylaxie visant à réduire l'incidence de la campylobactériose chez l'homme

J.A. Wagenaar, D.J. Mevius & A.H. Havelaar

Résumé

La campylobactériose est l'une des maladies d'origine bactérienne les plus importantes chez l'être humain. La transmission de l'infection à *Campylobacter* se fait principalement par contact direct avec les volailles et par ingestion de viande de volaille contaminée, mais d'autres sources existent et jouent un rôle parfois considérable. La prophylaxie de l'infection à *Campylobacter* chez les poulets de chair réduit efficacement l'incidence de la maladie chez l'homme.

Les poulets destinés à la consommation étant très facilement contaminés par *Campylobacter*, les mesures préventives dans les élevages ont des effets limités et imprévisibles. Les vaccins et les traitements recourant aux flores de barrière, aux bactériophages ou aux bactériocines ne sont pas encore disponibles sur le marché. Il est néanmoins possible de réduire la charge en *Campylobacter* dans le produit final en prenant des mesures appropriées à l'abattoir. La stratégie de prophylaxie la plus prometteuse à ce jour consiste à bien séparer, dans les abattoirs, les lots de volailles provenant d'élevages infectés de ceux provenant d'élevages indemnes. Ainsi, la viande de volaille issue d'élevages indemnes de *Campylobacter* peut-elle approvisionner sans risque le marché de viande fraîche, tandis que celle issue d'élevages infectés sera soumise à des traitements visant à limiter la charge en *Campylobacter*. La congélation est l'une des méthodes possibles, mais la décontamination chimique reste la méthode la plus rentable. Il est également possible de ne traiter que les viandes fortement contaminées.

La conclusion des auteurs est que, tant que de nouvelles méthodes ne seront pas disponibles, la gestion des lots à l'abattoir (autrement dit la stricte séparation des lots suivant le statut sanitaire de l'élevage d'origine) est la plus intéressante du point de vue économique. L'article s'achève sur une description de l'évolution de la résistance des *Campylobacter* aux antibiotiques.

Mots-clés
Abattage – Campylobacter – Gestion des lots à l'abattoir – Résistance aux antibiotiques – Santé publique – Sécurité sanitaire des aliments – Stratégie – Viande de volaille – Volaille – Zoonose.

El agente *Campylobacter* en la producción animal y las estrategias de control para reducir la incidencia de la campilobacteriosis humana

J.A. Wagenaar, D.J. Mevius & A.H. Havelaar

Resumen
La campilobacteriosis es una de las enfermedades bacterianas de mayor importancia transmitidas por los alimentos a los seres humanos. Si bien las principales fuentes de infección por *Campylobacter* son la manipulación y el consumo de aves de corral, también hay otras de considerable magnitud. El control del agente patógeno *Campylobacter* en los pollos de engorde reduce la incidencia de la campilobacteriosis humana.
El *Campylobacter* coloniza fácilmente los pollos para consumo y las medidas preventivas en la producción tienen efectos limitados e imprevisibles. Aún no se comercializan vacunas, productos para exclusión competitiva ni tratamientos con bacteriófagos o bacteriocinas. Pero, puede reducirse la contaminación del producto final mediante la aplicación de medidas de control en los mataderos. La estrategia de control más prometedora existente en la actualidad consiste en la matanza separada de las bandadas infectadas y sanas. La carne virtualmente libre de *Campylobacter* puede utilizarse para abastecer el mercado de productos avícolas frescos y la procedente de bandadas infectadas puede tratarse para reducir la concentración del agente patógeno. La carne de las bandadas infectadas puede tratarse por congelación, pero la descontaminación química parece más rentable. De igual modo es posible que se traten únicamente las carnes altamente contaminadas.
Los autores concluyen que mientras no se comercialicen técnicas nuevas, el procesamiento separado de las aves es el método de control más rentable. Por último, los autores describen la evolución de la resistencia antimicrobiana del género *Campylobacter*.

Palabras clave
Ave de corral – Campylobacter – Carne de pollo – Estrategia de intervención – Inocuidad de los alimentos – Matanza – Procesamiento separado – Resistencia antimicrobiana – Salud pública – Zoonosis.

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