CAMPYLOBACTERS AS ZOONOTIC PATHOGENS: A FOOD PRODUCTION PERSPECTIVE

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By Tom Humphrey, Sarah O'Brien and Mogens Madsen

CONTENTS

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EXECUTIVE SUMMARY

*C*ampylobacters remain highly important zoonotic pathogens worldwide which infect an estimated 1% of the population of Western Europe each year. Certain campylobacters are also important in infections of animals, particularly of the reproductive tract, and some are involved in periodontal disease.

This paper focuses, however, on the two species which are most important in food-borne infections of humans, *Campylobacter (C.) jejuni* and *C. coli*. Infection with these campylobacters is serious in its own right but can also have long term sequelae such as reactive arthritis and Guillain-Barré syndrome. The pathogens are ubiquitous in nature and in domestic animals and, as a consequence, are found frequently in the environment and on many raw foods, of both plant and animal origin and bacterial numbers can be very high on certain key foods like raw poultry meat. Although all commercial poultry species can carry campylobacters, the risk is greater from chicken because of the high levels of consumption. Campylobacters are relatively 'new' zoonotic pathogens as routine culture from clinical specimens only became possible in the late 1970s. As a consequence there is much that still needs to be understood about the behaviour and pathogenicity of these highly important bacteria.

In particular, and from a food industry/food safety perspective, it is important to better understand the behaviour of *C. jejuni* and *C. coli* in the food production environment, and how this affects their ability to survive certain food production processes. There is a belief that campylobacters are much more sensitive to hostile conditions than either salmonellas or *Escherichia coli*. Much of the data to support this view have been derived from laboratory experiments and may not fully represent the natural situation. Studies are showing that campylobacters may be more robust than previously thought and thus may represent a greater challenge to food safety.

We recommend that research is undertaken to better understand how campylobacters behave in the food chain and how responses to relevant conditions affect their ability to survive processing and their virulence. There is also a need to better understand the reasons why campylobacters are capable of frequent change, particularly in the expression of surface antigens.

INTRODUCTION

Campylobacters

The family *Campylobacteriaceae* comprises small (0.2-0.9μm wide and 0.2-5.0μm long), spiral formed (Figure 1), Gram-negative bacteria with 18 species, six sub-species and two biovars (Table 1). They are very different from other pathogens associated with food-borne disease in that they are essentially microaerophilic, growing best in an atmosphere containing approximately 10% CO_2 and approximately 5% O_2. The species pathogenic for man also have a rather narrow temperature range for growth with a maximum temperature of ~ 46°C and a minimum of 30°C. These are classed as thermophilic campylobacters.

Figure 1: A photomicrograph of C. jejuni *in the process of dividing*
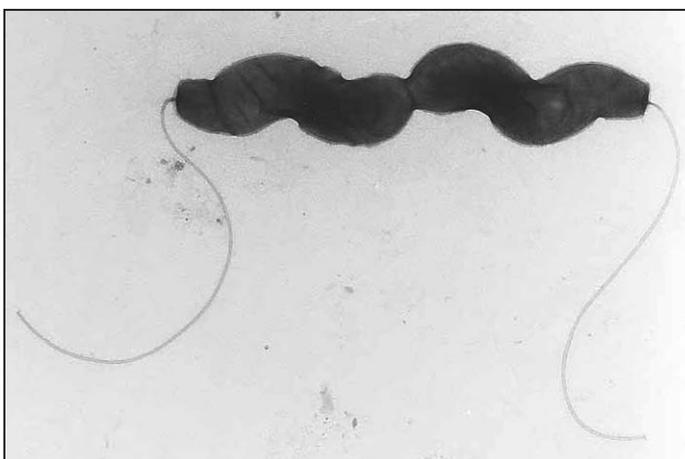
The above picture of *C. jejuni* was kindly donated by Dr. Mary Parker of the Institute of Food Research, Norwich, UK.

Table 1 shows the current members of the family *Campylobacteriaceae*. They are found in a wide range of sites in animals with some causing infections of the reproductive tract of certain domestic species which can lead to either abortion and/or infertility. Others are mainly involved in periodontal diseases. Campylobacters are principally known, however, as zoonotic pathogens. Most infections are caused by *C. jejuni* and *C. coli* although in the developing world *C. upsaliensis* is also important. *Campylobacter jejuni* and *C. coli* present an interesting dilemma. They can cause severe disease in infected people (see section on epidemiology) but are carried in the intestinal tracts of all types of domestic livestock and many wild animals, almost always without any harmful effects. This carriage does have major consequences for human health in terms of food borne disease. The differences in pathogen behaviour in man and in animals are not yet fully understood but are likely to be due to differential bacterial gene expression in different hosts.

Table 1: Current listing of members of the family Campylobacteriaceae

Family member	Known Source(s)	Disease associations	
		Human	**Veterinary**
C. coli	Pigs, poultry, cattle, sheep, birds	Gastroenteritis, septicaemia	Gastroenteritis
C. concisus	Man	Periodontal disease, gastroenteritis	None at present
C. curvus	Man	Periodontal disease, gastroenteritis	None at present
C. fetus subsp.*fetus*	Cattle, sheep	Septicaemia, gastroenteritis, abortion, meningitis	Bovine and ovine spontaneous abortion
C. fetus subsp. *venerealis*	Cattle	Septicaemia	Bovine infectious infertility
C. gracilis	Man	Periodontal disease, empyema, abscesses	None at present
C. helveticus	Cats, dogs	None at present	Feline and canine gastroenteritis
C. hyointestinalis subsp. *hyointestinali*	Pigs, cattle, hamsters, deer	Gastroenteritis	Porcine and bovine enteritis
C. hyointestinalis subsp. *lawsonii*	Pigs	None at present	Unknown
C. hyoilei	Pigs	None at present	Porcine proliferative enteritis
C. jejuni subsp. *doylei*	Man	Gastroenteritis, gastritis, septicaemia	None at present
C. jejuni subsp. *jejuni*	Poultry, pigs, cattle, sheep, dogs, cats, water, birds, mink, rabbits, insects	Gastroenteritis, septicaemia, meningitis, abortion, proctitis, Guillain-Barré Syndrome (GBS)	Gastroenteritis, avian hepatitis
C. lari	Birds (including poultry), water, dogs, cats, monkeys, horses, seals	Gastroenteritis, septicaemia	Avian gastroenteritis
C. mucosalis	Pigs	None at present	Porcine necrotic enteritis and ileitis
C. rectus	Man	Periodontal disease	None at present
C. showae	Man	Periodontal disease	None at present
C. sputorum bv. Sputorum	Man, cattle, pigs	Abscesses, gastroenteritis	None at present
C. sputorum bv. Faecalis	Sheep, bulls	None at present	None at present
C. upsaliensis	Dogs, cats	Gastroenteritis, septicaemia, abscesses	Canine and feline gastroenteritis
C. insulaenigrae	Seals, porpoises	None at present	None at present
C. lanienae	Cattle, pigs and humans	None at present	None at present
C. hominis	Humans	Gastroenteritis in the immunocompromised	

Disease incidence and clinical symptoms in humans

Campylobacters are the leading cause of bacterial diarrhoeal disease worldwide, although data are not yet available to allow an estimate of the contribution of these bacteria to all bacterial infections. The World Health Organization (WHO) estimates that ~1% of the population of Western Europe will be infected with campylobacters each year. This estimate is supported by data from England and Wales (Wheeler *et al.,* 1999), which found that for each reported case of campylobacter infection there were approximately nine others that were not reported. In England and Wales in 2004 there were ~50,000 cases reported. Assuming that the estimate of Wheeler *et al.* (1999) is correct, the true total is around 450,000, close to that suggested by WHO data. There are similarly high incidences throughout the developed world, but for unknown reasons the incidence is particularly high in New Zealand.

The infectious dose for campylobacters is low at a few hundred cells (Anonymous, 2005). Infection can have an incubation period of 1-10 days with most people exhibiting clinical symptoms by four days. It is characterised by profuse, often bloody diarrhoea, particularly in children, acute abdominal pain and fever. In the UK it has been reported that 82% of people admitted to hospital with a diagnosis of 'food poisoning' were suffering from campylobacter infection (Adak *et al.,* 2002). Most cases recover after a period of bed rest. As with other enteric infections maintenance of fluid balance is important.

Treatment with antibiotics for uncomplicated campylobacter infection is rarely indicated. However, antimicrobial resistance to clinically important drugs used for treatment (especially macrolides and fluoroquinolones) is increasingly reported for campylobacters. There is evidence that patients infected with antibiotic-resistant strains suffer worse outcomes (invasive illness or death) than those infected with sensitive strains (Helms *et al.,* 2005). This underlines the need to limit the use of antimicrobials in veterinary and medical clinical practice to limit the occurrence of resistance. In a small percentage of cases, long-term and potentially serious complications can arise. Infection with *C. jejuni* is the most common predisposing factor to the peripheral neuropathies Guillain-Barré (GBS) and Miller Fisher Syndromes. Not all strains of *C. jejuni* seem capable of causing these sequelae and there are differences in those associated with the two syndromes (Takahashi *et al.,* 2005). This is considered in greater detail later in this review.

This review will provide information on the epidemiology of campylobacters in human infection and food animal production. Potential control measures in chicken production are discussed in some detail as are possible post-processing treatments. Details are also provided on the behaviour of these pathogens in environments relevant to food production and how this might affect food safety.

We are aware that members of the genus *Arcobacter* are becoming increasingly recognised as zoonotic pathogens and that they have many behaviours and environmental niches in common with campylobacters. A definitive link between arcobacters and human disease has not yet been established but concern has been raised over their presence in meat and dairy products and over recent evidence suggesting that the genus *Arcobacter*, especially *A. butzleri*, may be involved in human enteric disease. The distinctive feature differentiating arcobacters from campylobacters is the ability of the former to grow at 15°C. Various aspects of arcobacters as potential food-borne pathogens have recently been reviewed by Lehner *et al.* (2005). This report concentrates on campylobacters but it is reasonable to assume that processes which control these bacteria in foods are likely to be equally successful against arcobacters.

EPIDEMIOLOGY OF CAMPYLOBACTER INFECTIONS IN HUMANS

Disease burden

The disease burden has been described in several different ways. In the US, it has been estimated that food-borne campylobacters infect around 2.5 million people each year (Mead *et al.,* 1999). In England and Wales, estimates show that there were approximately 360,000 cases of food-borne campylobacter infection in 2000, accounting for 27% of all food-borne disease (Adak *et al.,* 2002). In The Netherlands there are an estimated 80,000 cases per year (de Wit *et al.,* 2001) and the attributable cost of illness is approximately €21 million (Havelaar *et al.,* 2005). Thus the economic burden of campylobacter infection is large. The average cost of a case of acute campylobacter infection (excluding long-term sequelae) in England in 1995 was estimated to be €1,947 (£1,315). Conservatively, therefore, food-borne campylobacter infection costs the UK at least €96 million (£65 million) per annum and the true figure is probably closer to €740 million (£500 million) per annum.

In New Zealand in 1995 an economic appraisal put the annual cost of campylobacter infection at €2.57 million (NZ$4.48 million) (Withington and Chambers, 1997). In the US the annual estimated cost in the 1990s was around €3.52 billion (US$4.3 billion) (Buzby and Roberts, 1997).

Range and severity of symptoms and chronic effects

Classic symptoms of campylobacter infection include diarrhoea, which is frequently bloody, abdominal pain, fever, malaise, nausea and, rarely, vomiting. Complications of acute infection include intestinal haemorrhage, toxic megacolon and haemolytic uraemic syndrome. Mesenteric adenitis (inflammation of abdominal lymph nodes) can mimic acute appendicitis. The duration of illness is usually not longer than 10 days. Patients should be excluded from working as food handlers until they have been symptom-free for 48 hours but once this period is over there are no public health reasons to restrict a return to work. However, some food businesses may ask infected employees to submit stool samples for testing in line with their own occupational health requirements. In the longer term infection with campylobacters may lead to neurological and rheumatological sequelae. GBS is considered to be the most common cause of flaccid paralysis worldwide now that poliomyelitis is almost eradicated (Nachamkin, 2002). GBS and the non-paralytic variant Miller Fisher Syndrome are recognized sequelae of campylobacter infection (Nachamkin, 2002).

It is estimated that around 1 in 1,000 infections leads to GBS, the risk increasing to around 1 in 200 for patients infected with a particular *C. jejuni,* Penner type HS:19 (Nachamkin, 2002). There have been two main approaches to investigating the link between *C. jejuni* and GBS. The first follows cohorts of patients with *C. jejuni.* McCarthy and Giesecke (2001) found that the incidence of GBS in such patients in Sweden was 30.4/100,000, which is 100 times higher than in the uninfected population. In a follow-up study of cases of *C. jejuni* infection in Lancashire, England, Zia *et al.* (2003) showed that 11% of patients reported sensory problems and 9% weakness within four weeks of diarrhoea onset. An alternative approach is to investigate cohorts of patients with GBS and search for evidence of prior campylobacter infection. Estimates vary with the methods used but according to recent data the following percentages of GBS patients showed evidence of prior campylobacter infection:

1. 80% (based on serology) in The Netherlands (van Koningsveld, 2001);
2. 5% (stool culture) in India, which increased to 19% using PCR (Sinha *et al.,* 2004);
3. 11% (stool culture) in Japan (Takahashi *et al.,* 2005);
4. approximately 15% in England (Tam *et al.,* 2003).

It is considered that molecular mimicry of *C. jejuni* lipo-oligosaccharides (LOS) in gangliosides in nervous tissue induces cross-reactive antibodies that lead to GBS (Godschalk *et al.*, 2004).

Less is understood about mechanisms for reactive arthritis after *C. jejuni* infection. Following an outbreak in Finland 2.6% of those affected developed reactive arthritis with 33% being of a particular genetic type, as measured by human leucocyte antigen (HLA) (Hannu *et al.,* 2004). In a Swedish cohort of patients with recent-onset arthritis 45% had evidence of previous campylobacter infection (Soderlin *et al.,* 2003). In two recently published studies following cohorts of patients with *C. jejuni* infection 7% of the Finnish patients (Hannu *et al.,* 2002) and 7% of those from Lancashire, UK developed reactive arthritis (Zia *et al.,* 2003). In the Lancashire study 37% of patients overall complained of musculoskeletal problems, whilst in Finland a further 1% of patients presented with reactive tendonitis, enthesopathy or bursitis.

Finally, it has been estimated that around 25% of post-infectious irritable bowel syndrome may be attributable to campylobacter infection (Neal *et al.,* 1997).

Disease Trends

Table 2 shows campylobacter incidence rates for some countries in Europe for 1993-2000. The increase in laboratory-confirmed cases recorded by national surveillance bodies in a range of countries may have peaked, although this is not a universal observation. In the US the number of cases recorded in FoodNet started to fall in 1996 (Samuel *et al.*, 2004). Data on disease trends in the US can be found on CDC FoodNet, - at: http://www.cdc.gov/ncidod/dbmd/diseaseinfo/campylobacter_t.htm. Since 2000 the number of cases in England, Wales, and Denmark (see http://www.ssi.dk/germ/) also fell. No evidence exists to suggest that surveillance methods have changed markedly and the fall in the number of cases may be real. It is too soon, however, to be certain of this and caution needs to be exercised in the interpretation of such data.

Seasonality

A prominent characteristic of campylobacter epidemiology is its marked seasonality. In temperate climates, incidence peaks in late spring/early summer. In North West England surveillance of campylobacteriosis showed a peak of cases in May (Sopwith *et al.*, 2003). In Scotland, the annual peak is in late June/early July and is more evident in rural/semi-rural than urban areas (Miller *et al.*, 2004). A European study (Nylen *et al.*, 2002) showed that the timing of the seasonal peak varied, occurring earlier in Wales (weeks 23-27) than in Scotland (weeks 24-27) and the Nordic countries (weeks 29-35). Finally, in New Zealand there is a marked difference in the seasonality between the North and South Islands (Hearnden *et al.*, 2003). Several hypotheses have been put forward to explain this seasonality, including:

Climate: Recent studies showed a correlation between ambient temperature and the number of cases. In Denmark, Patrick *et al.* (2004) demonstrated that the maximum temperature four weeks prior to infection is the best predictor of human cases. However, in this study only the effects of four climatic parameters (temperature, precipitation, relative humidity and hours of sunlight) were analysed. In an international study, Sari Kovats *et al.* (2005) found a weak association between case occurrence and ambient temperature. Finally, Louis *et al.* (2005) have shown a strong correlation with ambient temperature, the effect being most marked in children under the age of 5 years.

Table 2: Incidence rates of campylobacteriosis in selected European Countries 1993-2000

Country	1993 No of Cases	1993 Incidence Rate	1994 No of Cases	1994 Incidence Rate	1995 No of Cases	1995 Incidence Rate	1996 No of Cases	1996 Incidence Rate	1997 No of Cases	1997 Incidence Rate	1998 No of Cases	1998 Incidence Rate	1999 No of Cases	1999 Incidence Rate	2000 No of Cases	2000 Incidence Rate
Finland	1600	31	1804	35	2197	42	2629	51	2404	46	2851	46	3303	64	3527	68
Iceland	59	21	48	17	41	15	85	31	93	4	220	80	435	156	245	87
Norway	877	20	1050	24	1046	24	1145	26	1178	27	1700	39	2032	46	2331	52
Sweden	4485	21	5529	29	5580	29	5081	21	5306	21	6543	29	7137	81	7646	86
Belgium	4394	44	4879	48	4779	47	4991	49	5417	54	6610	65	6514	64	7473	73
Netherlands	N/A	N/A	N/A	N/A	2871	18	3741	24	3646	23	3398	22	3160	N/A	3362	N/A
U.K. England & Wales	39477	74	45207	84	43449	83	43978	82	51360	95	56852	110	54987	104	55887	106
U.K Scotland	4011	78	4152	80	4381	86	5107	102	5533	108	6381	125	5865	115	6482	127
Switzerland	5058	71	4931	69	544	71	5656	79	5955	84	5455	77	6709	94	7568	105

N/A = Not Available;
Source: WHO surveillance programme for control of foodborne infections and intoxications in Europe 7th and 8th Reports
(http://www.euro.who.int/foodsafety/surveillance/20031127_1);
Health Protection Agency (http://www.hpa.org.uk/infections/topics_az/campy/data_ew.htm);
Health Protection Scotland (http://www.show.scot.nhs.uk/scieh/).

Poultry and other food-producing animals: It has been suggested that the peak in human cases might relate to fluctuations in carriage in poultry and other food-producing animals. However, available evidence does not consistently support this hypothesis. In Finland, Karenlampi *et al.* (2003) found an overlap of 34% between sero-/genotype combinations in sporadic *C. jejuni* infections and those in chicken flocks at slaughter during a seasonal peak. In Wales, Meldrum *et al.* (2005) showed that human infections peak before campylobacter contamination in fresh, retail chicken. Patrick *et al.* (2004) found that average and maximum temperatures three weeks prior to slaughter were the best climatic predictor of broiler flock carriage. Wallace *et al.* (1997) found that seasonal fluctuation of campylobacters in chickens correlated with hours of sunshine and minimum and maximum temperatures. The periodicity of carriage in caeca was different from that in the small intestine of the birds. Significant seasonal variation has also been shown to occur in the shedding of thermophilic campylobacters in fresh faeces from dairy cattle (Stanley *et al.*, 1998). The pattern roughly coincided with the human seasonal peak, although a formal statistical relationship was not tested in this study.

Migratory wild birds: Pacha *et al.* (1998) screened Canada geese, migratory ducks and sandhill cranes for campylobacters and found high carriage rates of *C. jejuni* in all species, concluding that wild birds may play a role in spreading the organism in the environment. However, Broman *et al.* (2004) suggested that strains in wild birds are largely different from those in humans on the basis of Pulsed Field Gel Electrophoresis (PFGE) analyses.

Companion animals: Evans (1993) suggested that the seasonal peak in human campylobacter infection reflected the seasonality of canine births and that more puppies acquired as pets in the summer months might contribute to the human disease burden.

Flies: Hald *et al.* (2004), Nichols (2005) and Ekdahl *et al.* (2005) have recently hypothesized that the seasonal peak in humans might be explained by flies acting as either mechanical or biological vectors.

Epidemiological evidence that campylobacter infection is food borne

Evidence that campylobacter infection is food borne comes from two main sources: investigations of outbreaks and analytical, epidemiological studies of sporadic disease.

Investigations of outbreaks

One feature of campylobacter infection is that general outbreaks (affecting members of more than one household) are rarely recognised (Pebody *et al.*, 1997; Frost *et al.*, 2002). In 1995-1999 374 general outbreaks of infectious intestinal disease in England and Wales were reported to the Health Protection Agency Centre for Infections (HPA CfI). Where an agent was identified, campylobacters accounted for 50 (2%) of them (Frost *et al.*, 2002). Cross-contamination was the most commonly reported food-handling fault (18 outbreaks) (Frost *et al.*, 2002). The proportion of campylobacter cases, recognised as part of outbreaks in this period, was only 0.4% compared with 8% for salmonellas and 15.5% for *E. coli* O157 (Frost *et al.*, 2002). Thirty-five of the 50 outbreaks reported to HPA CfI between 1995 and 1999 were food borne. Where a food vehicle was identified (24/35 outbreaks) the most frequent was poultry (13 chickens, one duck). In a study of gastroenteritis outbreaks in The Netherlands campylobacters were identified in 1% of 281 (van Duynhoven *et al.*, 2005).

A large sentinel survey of apparently sporadic campylobacter infections in England suggested that point source general outbreaks might be more common than is currently recognised. Of the 3,489 cases of *C. jejuni* infection in the first year of the study 333 (10%) reported knowledge of an individual outside the household with a similar coincident illness (Gillespie *et al.*, 2003a). Subjects

who reported other illness in the community were more likely to have eaten in restaurants or consumed unpasteurised milk. These findings were consistent with previous studies on the risks from unpasteurised milk (Gillespie *et al.*, 2003b) and the impact of the restaurant setting in campylobacter outbreaks (Pebody *et al.*, 1997; Frost *et al.*, 2002).

Sporadic disease

Risk factors for sporadic disease (cases not caused by outbreaks and comprising the majority) have been sought in case-control studies conducted in various settings. A particular strength of such studies is that they are efficient ways of testing multiple hypotheses regarding routes of transmission and vehicles of infection. Most are retrospective and rely on cases and controls remembering accurately what they have done and/or eaten. The longer the time between illness and questioning the less reliable are peoples' memories. Poor case and control selection, or high refusal rates, can also limit study findings. In seeking to explain sometimes conflicting findings from case-control studies it is useful to recognise that this type of enquiry only identifies food vehicles. These might, but need not be, the same as the contamination source. Chicken contaminated with campylobacters might be how organisms enter the kitchen and might lead to cross contamination of, for example, lettuce. If the chicken is cooked properly it is unlikely to be implicated as a food vehicle in a case-control study, yet eating the lettuce, which has not undergone any further cooking, might well be identified as a risk factor.

Nevertheless, good case-control studies can provide important clues about the origins of human infections. Such studies have demonstrated the complex epidemiology of campylobacter infection and, each time, a range of exposures has been identified:

1. *Poultry:* Consumption of poultry has been identified as a risk factor in several studies as summarised below:
   - any type of chicken (Norkrans and Svedhem, 1982; Oosterom *et al.*, 1984; Deming *et al.*, 1987; Neal and Slack, 1997; Studahl and Andersson, 2000);
   - poultry and poultry liver (Schorr *et al.*, 1994);
   - raw or under-cooked chicken (Hopkins *et al.*, 1984; Harris *et al.*, 1986a; Neimann *et al.*, 2003; Friedman *et al.*, 2004; Michaud *et al.*, 2004);
   - cooked chicken (Harris *et al.*, 1986b);
   - processed chicken (Klatka *et al.*, 2002);
   - barbecued chicken (Ikram *et al.*, 1994; Adak *et al.*, 1995);
   - chicken prepared by or eaten in a commercial food establishment (Eberhart-Phillips *et al.*, 1997; Effler *et al.*, 2001; Rodrigues *et al.*, 2001; Klatka *et al.*, 2002; Friedman *et al.*, 2004, Michaud *et al.*, 2004).

In a case-control study of primary, indigenous, sporadic campylobacteriosis in England and Wales consumption or handling of chicken cooked and eaten in the home was found to be protective (Adak *et al.*, 1995). The term 'protective' when used is this context means that people who regularly eat and prepare chicken at home have a lower rate of infection than those who do not. Similarly, in a study in New Zealand, recent consumption of baked or roast chicken seemed to be protective, although consumption of raw or undercooked chicken, or chicken from restaurants was associated with illness (Eberhart-Phillips, 1997). An earlier study in New Zealand also showed that eating at home was protective (Ikram *et al.*, 1994).

2. *Other foods:* Other foods implicated as risk factors for sporadic infection include:
- barbecued/grilled meat (Deming *et al.*, 1987; Kapperud *et al.*, 1992; Kapperud *et al.*, 2003; Neimann *et al.*, 2003; Carrique-Mas *et al.*, 2005). Where meat type is described, red meat (unspecified) and sausages have been implicated;
- undercooked meat (Schonberg-Norio *et al.*, 2004);
- raw milk (Saeed *et al.*, 1993; Schorr *et al.*, 1994; Eberhart-Phillips *et al.*, 1997; Studahl and Andersson, 2000; Neimann *et al.*, 2003; Michaud *et al.*, 2004);
- bird pecked milk (Lighton *et al.*, 1991; Neal and Slack, 1997);
- bottled mineral water (Evans *et al.*, 2003);
- salad vegetables (Evans *et al.*, 2003; Karenlampi *et al.*, 2003);
- grapes (Neimann *et al.*, 2003).

3. *Water:* Exposure to the following have been associated with a significantly increased risk of developing campylobacter infection:
- consumption of untreated water (Schorr *et al.*, 1994; Klatka *et al.*, 2002; Endtz *et al.*, 2003; Kapperud *et al.*, 2003; Schonberg-Norio *et al.*, 2004);
- consumption of rainwater (Eberhart-Phillips *et al.*, 1997);
- having a household well (Carrique-Mas *et al.*, 2005);
- consumption of recreational water (river/lake water) (Schonberg-Norio *et al.*, 2004; Carrique-Mas *et al.*, 2005).

A study in Sweden identified positive associations between campylobacter infections and on the one hand average water-pipe length per person and on the other hand density of ruminants, suggesting that drinking water and contamination from livestock might also be important factors in explaining at least part of the burden of human sporadic campylobacteriosis (Nygard *et al.*, 2004).

4. *Other risk factors:* In addition to risks from food and water consumption the following have been shown to be associated with an increased risk of campylobacter infection:
- contact with either domestic pets or farm animals, including occupational exposure (Kapperud *et al.*, 1992; Saeed *et al.*, 1993; Schorr *et al.*, 1994; Studahl and Andersson, 2000; Klatka *et al.*, 2002; Potter *et al.*, 2003; Wilson, 2004; Carrique-Mas *et al.*, 2005);
- problems with the household sewage system (Eberhart-Phillips *et al.*, 1997);
- underlying medical conditions like diabetes (Neal and Slack, 1997) or reduced gastric acidity due to the use of proton pump inhibitors (Neal *et al.*, 1996).

It should be noted that in the majority of case–control studies reported to date, recognised risk factors rarely explain more than around half of the cases. Adak *et al.* (2005) estimated the proportion of food borne campylobacter infection that might be due to various types of foods consumed. They concluded that the most important cause of acquired food-borne disease in the UK was contaminated chicken leading to 398,420 cases of illness and representing a risk of 111 cases/million servings. The influence of campylobacter infection contributed heavily to this risk estimate.

Supporting the poultry hypothesis

There is no doubt that poultry is a major source of campylobacters (Jørgensen *et al.*, 2002) and there is scope for cross-contamination of other foods if contaminated poultry is introduced into the kitchen. Two additional pieces of evidence support the thesis that poultry is an important source of human campylobacter infection. The first comes from Belgium and occurred when Belgian poultry and eggs were withdrawn in May/June 1999 because of contamination with dioxins (Vellinga and van Loock, 2002). There was a coincident 40% reduction in human campylobacter cases. The second piece of evidence comes from Iceland. In common with many other Nordic countries chicken was sold frozen in Iceland prior to 1996. However, increased consumer demand for poultry and market driven pressures led to the sale of chilled chicken after 1996. Following this, human campylobacter infections increased and peaked in 1999, at a rate of 116/100,000. At this time 62% broiler carcass rinses were positive for campylobacters. A number of preventative measures were introduced, including improving biosecurity on farms, freezing of birds from flocks testing positive at one week before slaughter and public education. In 2000 human infection dropped to 33 cases/100,000 and only 15% of broiler carcass rinses were campylobacter-positive (Stern *et al.*, 2003). No specific measure was identified as contributing to the fall in cases but the combination of measures was effective.

Campylobacter infection is a major public health problem with complex epidemiology, extensive animal and environmental reservoirs and multiple risk factors. Although epidemiological patterns, such as seasonality, are well described their underlying explanations remain obscure. Poultry is an important source of infection and eating food, including poultry, on commercial catering premises has been identified as a risk factor in several case-control studies. However, many studies also point to numerous other sources and vehicles of infection and it is important that these are not overlooked.

DETECTION, ISOLATION AND TYPING

Traditional methods

Many methods for isolating campylobacters from clinical specimens have been published and Bolton *et al.* (1997) showed that they can be isolated from human faecal samples using microaerobic-atmosphere-generating systems. With foods enrichment is usually required. The broth used affects recovery (Baylis *et al.*, 2000) and the sampling method affects the numbers recovered (Jørgensen *et al.*, 2002). The time to confirm the presence of campylobacters in food and environmental samples can exceed five days, and presents difficulties particularly in outbreak investigation and for positive release. Positive release involves testing the products to show that they are pathogen-free before they are put in the food chain. Attempts have been made to reduce isolation/confirmation times and a variety of kits is available. There is debate about isolation methods for foods and water but, as with salmonellas, it is likely that no single method is ideal for the entire range of foods requiring testing. Data suggest that Bolton broth (see ISO literature, Figure 2) gives the highest isolation rates.

Figure 2: Diagram of ISO procedure for isolation of Campylobacters from food

Test portion

x g or *x* ml

↓

9*x* g or 9*x* ml Bolton broth (5.2)*

↓

ENRICHMENT

Incubation in a microaerobic
atmosphere at 37 °C for 4 h to 6 h and
then at 41.5°C for 44 h ± 4 h

↓

ISOLATION

mCCD agar (5.3)*
+
2nd medium, as preferred

↓

Incubation in a microaerobic
atmosphere at 41.5°C for 44 h ± 4 h

↓

Characteristic colonies (9.4.2)*

↓

Confirmation (9.4.3 to 9.4.6)*

↓

Identification (optional) (9.5)*

↓

Expression of results (Clause 10)*
and test report (Clause 11)*

The terms and definitions taken from ISO 10272-1:2006 Microbiology of food and animal feeding stuffs – Horizontal method for detection and enumeration of Campylobacter spp. – Part 1, Annex A, Diagram of procedure, are reproduced with permission of the International Organization for Standardization, ISO. This standard can be obtained from any ISO member and from the Web site of ISO Central Secretariat at the following address: www.iso.org. Copyright remains with ISO.

* numbers in parenthesis are section numbers in the ISO document

Immuno-based assays

Campylobacters elicit antibody responses in infected hosts and these proteins have the potential to be used for rapid detection and/or confirmation of the pathogen in foods. A group in Ireland (Grennan *et al.*, 2001) described a PCR-ELISA for the detection of campylobacters and the discrimination of *C. jejuni* and *C. coli* in poultry samples. The PCR assay targeted the 16S/23S ribosomal RNA intergenic spacer region of campylobacters with DNA oligonucleotide probes. Their studies showed that PCR-ELISA, when combined with culture pre-enrichment, was able to detect the presence of campylobacters and definitively identify *C. jejuni* and *C. coli* in culture-enriched poultry meat samples.

DNA-based detection methods

DNA-based detection methods, such as PCR, are available for campylobacters. In contrast to classical culture, detecting living bacteria that can grow, genome-based methods detect DNA from live and dead bacteria. This poses no problems when the samples are fresh faeces or caecal contents with large numbers of viable campylobacters but presents serious interpretation difficulties when used for analysis of environmental and food samples where both live and dead bacteria are likely to be present. For example, cooked chicken will contain substantial numbers of dead campylobacters. This limitation of molecular methods is not restricted to campylobacters and is also relevant for other micro-organisms.

Polymerase chain reaction (PCR) detection methods are directed at short fragments of the genome that may be multiplied and visualised following electrophoresis in an agar gel, for example. Depending on the specificity different regions of the genome may be targeted. For the detection of the genus *Campylobacter* a highly conserved region such as the 16S rRNA is the target for the PCR , while more specific loci are used for the detection of particular species. One example is the hippurate gene for *C. jejuni* (Bang *et al.*, 2002). Since 2001 PCR detection has been used extensively in the National Campylobacter Surveillance Program in Denmark (Lund *et al.*, 2003).

Conventional PCR detection can be demanding on technical staff due to the experience needed to prevent cross-contamination with amplified DNA. For this and other reasons Real-time (RT) PCR has been developed (Lund *et al.*, 2004, Yang *et al.*, 2005). With this technique, the multiplication of DNA fragments can be followed as a rising curve develops (Sails *et al.*, 2003). This method is less demanding on the technical staff and can be performed by people with less experience. In addition, this technique adds a quantitative dimension to detection, as the number of cycles required to reach the threshold value is directly correlated with the initial numbers of campylobacters in the sample, if not pre-enriched.

A drawback of PCR-based detection methods, but one shared with other methods, is that many laboratories have developed specific protocols that work well in their own laboratory but do not allow comparison of results between centres using different protocols. For this reason inter-laboratory proficiency tests, collaborative trials and standardised protocols are much needed (Josefsen *et al.*, 2004). Several PCR-based kits for the detection of campylobacters in foods are commercially available (Wang, 2002). Much attention has been paid to the development of rapid methods for the end of the isolation process and rather less to improving the growth rates and recovery of campylobacter cells in the hours following inoculation of the primary broth. Work is needed in this area, particularly on the optimisation of recovery media and incubation conditions and on the balance between selectivity and sensitivity.

Typing

There is still much debate about the best methods for distinguishing one strain of campylobacter from another and/or to trace sources in outbreaks. It is not our intention to give a detailed discussion of this topic in the present report. Traditional typing methods are phenotypic, particularly using differences in the structure of key surface antigens such as LPS or flagella. The typing scheme for salmonellas has very successfully used this approach for many decades. Such methods do not appear to be as useful with campylobacters, as their surface structures can be highly variable, even within an individual strain. It is likely that in the future the typing of campylobacters will be achieved using genome-based methods. The UK Advisory Committee for the Microbiological Safety of Food (ACMSF) recently published a review of campylobacters as zoonotic pathogens (Anonymous, 2005) and this includes a detailed section on typing. The most recent developments of molecular methods for typing campylobacters include multi-locus sequence typing (MLST) and DNA microarrays.

Multi-locus sequence typing (MLST), which has been used very successfully to study population structure in Neisseria, is increasingly being used with campylobacters. This technique compares DNA sequence differences in seven campylobacter house-keeping (essential) genes using the technical approaches described above (Dingle *et al.*, 2002). This method has allowed the identification of different clonal groupings of *C. jejuni* and has shown that particular clones are found in specific animals, although some are more widespread and also found in humans. A strength of MLST is that it is subject to much less variation than phenotypic methods. This is also a potential weakness, however, because certain common clones can require further differentiation. This has been achieved by adding two other genes involved in the synthesis of flagella proteins to the analysis as they are more variable.

The potential for PCR detection to double as a tool for strain characterisation has recently been emphasised by Best *et al.* (2005). They have developed real-time PCR Taqman allelic discrimination assays designed to detect the single nucleotide polymorphisms specific for six major MLST clonal complexes allowing the rapid detection of *C. jejuni* isolates and preliminary strain identification.

Finally, the full sequencing of the *C. jejuni* genome (Parkhill *et al.*, 2000) has opened up the 'post-genomic era', aiming at the detection of specific genes thought to be important for the pathogenesis of campylobacters. Thus, present research efforts are now directed at DNA microarrays and down-scaling of detection processes to the 'Lab-on-a-chip' scale (Keramas *et al.*, 2003, 2004). These methods may prove to be very rapid and also have the potential to handle a large number of samples, analysing for a number of parameters simultaneously.

CAMPYLOBACTERS IN PRIMARY FOOD PRODUCTION

*A*ll animals used for food can be campylobacter-positive as can many companion species (domestic pets). Samples from the natural environment, such as groundwater (Schaffner *et al.*, 2004), will also frequently contain these pathogens. The Schaffner study reports the presence of campylobacters in groundwaters of mainly mountainous regions. The authors concluded that these organisms multiply in a natural way in the environment or that they are able to survive for a long time. The greatest current risk to human health, however, is posed by contaminated chicken and the present report will focus on that animal. Although all commercial poultry species can carry campylobacters the risk is greater from chicken because of the large quantities consumed. For example, in the UK ~ 800 million chickens are consumed annually.

Table 3 gives details of some published information on the incidence of campylobacters in food animals, which confirms that these human pathogens are commonly found in many types of animals used for food. The risks to human health vary between the different animal species and will also be different between countries often due to variations in food preparation and consumption patterns.

Table 3: Isolation of campylobacters from raw foods and food animals

Food or animal tested	Mean % positive samples	% Range
Dairy cows	30.0	6-64
Beef cattle	62.1	42-83
Sheep	31.1	18-44
Pigs	61.0	50-69
Chicken flocks	58.7	2.9-100
Turkey flocks	78.0	20-100
Duck flocks	38.0	0-88
Raw milk	3.2	0-9.2
Chicken at retail	57.4	23-100
Turkey at retail	47.8	14-94
Duck at retail	30.2	19-46
Pork at retail	2.0	0-5.1
Beef at retail	2.7	0-9.8
Lamb at retail	6.0	0-12.2

*Data compiled by Tom Humphrey based on publications from 21 different countries.

Zoonotic campylobacters in cattle, sheep and pigs

This section will concentrate on *C. jejuni* and *C. coli*, both commonly found in cattle, sheep and pigs (Stanley and Jones, 2003; Nielsen, 2002; Payot *et al.*, 2004; Boes *et al.*, 2005). It is believed that these animals acquire the organisms by contact with a contaminated environment. Humphrey and Beckett (1987) demonstrated a link between the consumption of water from natural sources and the presence of campylobacters in dairy cows. Most animals in a herd will carry these organisms, although carriage levels will vary between individuals and some will not even be colonised at all. As with many issues in the epidemiology of campylobacters in animals and man the reasons for this variation are not known. They may well reflect differences in gut commensals and/or immunity and require investigation. Such observations could also indicate that campylobacters may not be natural gut commensals like *E. coli* or faecal streptococci, for example. The commonality of carriage may reflect frequency of challenge from the environment and cycling between individuals in a herd. Most cattle and sheep are reared in outdoor systems where there will be frequent contact with the external environment. Free-range systems are also becoming increasingly common in pig production. No measures have yet been identified which will protect outdoor-reared animals from infection with campylobacters. At present, and for the foreseeable future, control must be applied later in the food chain. This largely revolves around improving hygiene at milking and slaughter and particularly pasteurisation of milk.

Contamination of dairy products

The presence of campylobacters in the intestinal tract of dairy animals will mean that milk will frequently be contaminated at milking as a consequence of faecal contamination. Although proper hygiene at milking can reduce both the incidence and level of contamination, and udders should be washed and dried prior to milking, this is not a completely effective control measure. The only way to ensure that people are protected from infection by this route is for milk to be pasteurised, as this process, if applied properly, will kill campylobacters. There have been outbreaks caused by pasteurised milk but this is associated with either contamination with raw milk after pasteurisation or incorrectly applied heat. There are also very few, if any, known instances of fermented dairy products causing outbreaks of campylobacter infection. Even though *C. jejuni* can mount an acid tolerance response (see below) it has been shown to survive poorly in both cheese (Bachmann and Spahr, 1995) and yoghurt (Cuk *et al.*, 1987). In fact, rather than pose a risk of campylobacter infection, the consumption of yoghurt has been shown to be protective in a study in Switzerland (Schorr *et al.*, 1994).

Red meat as potential vehicles for transmission

Red meat animals can very often arrive campylobacter-positive at slaughter. It has been shown that levels of excretion of campylobacters can be higher in animals after transport and this is associated with stress in the host animal. Carcasses may become contaminated by spillage of faecal material, but this can be reduced by good slaughtering hygiene. Table 3 shows that, in general, the frequency of contamination of red meat products at retail is lower than that seen in poultry. The slower rate of slaughter in red meat abattoirs will be a factor in this. The most important reason for the differences between red and white meat, however, is that carcasses of the former will be subjected to an extended chilling prior to entry into the food chain. Of the many stresses experienced by these pathogens in food production, desiccation appears to be the most damaging and campylobacters survive poorly on dry surfaces (Humphrey *et al.*, 1995). This means that when red meat carcasses are chilled the numbers of campylobacters present on exposed surfaces will be markedly reduced by drying. Although this treatment is not a hundred percent effective it does mean that the risk posed by red meat is less than that associated with poultry, and this is reflected in the epidemiology of campylobacters in human infection. There is nevertheless a risk associated with red meat and care is still required in household kitchens and particularly in commercial catering as illustrated by data from the USA (Friedman *et al.*, 2004).

There is relatively little information on the survival of campylobacters in fermented meats. One study (Bostan *et al.*, 2001) which examined Turkish fermented sausages reported relatively rapid die-off (by day four of the fermentation). This may also be true for other fermented meats but a lack of data/studies makes it difficult to ascertain this hypothesis.

Fresh produce

Ready-to-eat fresh produce contaminated with enteric pathogens presents a risk to consumers. However, its importance as a source of campylobacters is unclear. The number of documented food borne outbreaks associated with raw fruits, vegetables and unpasteurised fruit juices has increased (Buck *et al.*, 2003). Such foods can present a campylobacteriosis risk to public health as a consequence of using contaminated irrigation or washing water. Preventing contamination of fresh produce with campylobacters relies on the application of good agricultural practice (GAP) and good hygienic practice (GHP) throughout the production chain. Important control options include not using untreated faecally contaminated water for irrigation and washing (Anonymous, 2004). In some outbreaks food handlers or cross-contamination in the kitchen have been shown as sources of contamination of fresh salads.

Campylobacters in poultry production

The importance of chicken as a source and vehicle of human infection with campylobacters cannot be over-emphasised. Data in Table 3, and elsewhere in this report, demonstrate that not only are many (~ 60%) chickens entering household and commercial kitchens (and food manufacturing facilities) campylobacter-positive but also that contamination levels, in terms of bacterial numbers on carcasses, can be extremely high constituting many infectious doses. There is an urgent need to reduce both the incidence and levels of carcass contamination. In theory intervention is possible at many points along the production chain. We will concentrate on on-farm aspects of control, as this is likely to be most cost-effective, although consideration will also be given to the possibility of intervention during poultry processing. This section focuses on chicken production, because chicken meat has by far the largest market share of all poultry, but campylobacters can be found in all commercial poultry species, including chickens, ducks, geese and turkeys (Table 3). Furthermore, most is known about intervention measures in chicken production, but many of these interventions will be applicable to other birds. Chickens are mainly reared using the broiler system where birds are housed and given food and water *ad libitum*. Growth rates of the birds are rapid so they attain slaughter weight in around 40 days.

Seasonal trends

There is a distinct seasonality in the occurrence of campylobacter-colonised broiler flocks (Wedderkopp *et al.*, 2001; Anonymous, 2005; Meldrum *et al.*, 2005; Ekdahl *et al.*, 2005). Seasonal variation is also seen in human campylobacteriosis (see the section on epidemiology). In the northern hemisphere the number of campylobacter-positive poultry flocks can be low in winter, and the likelihood of this increases the further north birds are farmed, while the prevalence rises sharply to reach a maximum in July-August. However, demonstrating synchronicity of poultry and human infections is not straightforward. In a recent comparative study temporal associations were not demonstrated and the authors concluded that the seasonal rise of infections in humans is not caused by the concurrent increase in poultry contamination but that both are more likely to be associated with a common, as yet unidentified, environmental source (Meldrum *et al.*, 2005). The seasonal pattern suggests that different mechanisms of introduction operate during summer and recent evidence points to flies as potentially important vectors of infection for poultry flocks during this period (Hald *et al.*, 2004, Nichols, 2005, Ekdahl *et al.*, 2005). The insects could be carried into houses by increased ventilation airflow during warmer weather.

Routes of infection for poultry

There is a large body of data available on sources of infection of broilers with campylobacters. The importance of the different routes indicated below will vary between countries:

1. **The external environment around the broiler house and domestic and/or wild animals:** The external environment is thought to be the most important source of campylobacters. The bacteria can be common contaminants of the farm environment and types from this source have been shown to be identical to those isolated from birds. It is likely that the bacteria come from wild and domestic animals. Farms with mixed animal species also run the risk of increased flock infection because campylobacter strains found in cows and pigs can also be isolated from chickens. Ideally, chickens should be reared on farms devoted to them only. Pets are frequently campylobacter-positive and should not be allowed access to poultry flocks. Surveys in a number of countries have shown that 19% (range 0.8-41) of cats and 32% (range 11-50) of dogs are campylobacter-positive. As mentioned above houseflies have also been shown to act as a source of *C. jejuni* and Rosef and Kapperud (1983) isolated campylobacters from around 50% of flies sampled on a chicken farm.

2. **Contaminated water:** Measures, which allow campylobacters to enter poultry houses place flocks at risk of campylobacter infection. One of these is the use of contaminated water. Investigations in the UK and Scandinavia have shown that this can introduce campylobacters into poultry flocks. This is particularly true if water of non-potable quality is used. In theory, waterborne infection should be the easiest to control as it may require only the addition of treatment chemicals, and it is very important that flocks receive only water of potable quality. Water may also act as a vehicle for horizontal transmission within the broiler house once campylobacters have become established. Bell drinkers, which have an open body of water that allows chickens direct access to the water, can be important in this respect.

3. **Flock thinning, house clearance and transport:** The generally very poor economic returns for poultry farmers mean that measures are adopted to maximise income. One is "thinning". The number of birds that can be placed into a broiler house is legally limited for welfare reasons to avoid the space becoming too crowded as the birds approach slaughter weight. To increase the economic return broiler houses can be stocked with more birds than is allowed provided the excess number is removed before welfare limits (based on weight per square metre) are exceeded. Thus, on many farms, the broiler houses are filled with around 130% of the final stocking density. Approximately seven days before slaughter the extra birds are removed for slaughter. Studies in Denmark found that thinning significantly increases the risk of campylobacter infection of the birds remaining in the flock (Hald *et al.*, 2001) because it compromises biosecurity by catching personnel breaking the hygiene barriers during collection of birds. Thinning also has serious implications for bird welfare. Unless there is an increase in the income farmers receive for their chickens thinning will continue and for public health reasons this practice should be made as hygienic as possible. The crates, vehicles and modules used to transport the birds to the processing plant should be campylobacter-free although this is very difficult to achieve in current commercial practice. Many of the problems identified with thinning will also occur at final clearance of the broiler house, particularly on multi-house sites, which can require many days to remove all the birds.

4. **Carry-over from a previous flock:** This is essentially another biosecurity (see below for a definition of this term) issue and is suggested to be a consequence of ineffective cleaning and disinfection of broiler houses between one flock and the next. Evidence for carry-over comes from studies showing that the 'same' campylobacters can be isolated from successive flocks. This could also be caused, however, by repeated introductions from a common source in the

environment outside the chicken house. Campylobacters are thought to be more sensitive to cleaning and disinfection than salmonellas, for example, although this view may have to be changed as we learn more about the former. It is likely that a cleaning and disinfection regimen which removes salmonellas will also destroy campylobacters. It is important that house cleaning and disinfection are carried out effectively.

5. **Vertical transmission from parent flocks:** The currently most contentious area in the epidemiology of campylobacters in chicken production is the possibility that the bacteria may be transmitted from infected breeding flocks in a manner similar to that of *S.* Enteritidis. Such transmission is a result of infection of reproductive tissues and associated contamination of eggs. It has been suggested that, because a small minority of flocks can be campylobacter-positive within a week of hatching, this route is possible. *Campylobacter jejuni* can be recovered from the oviduct, which suggests a possibility of egg contamination and it has also been found in semen samples from breeder cockerels. It has yet to be shown unequivocally, however, that campylobacters can be isolated from newly hatched chickens which would be the ultimate test of vertical transmission. The fact that housed birds do not generally become infected until around four weeks of age is an argument against vertical transmission, although it cannot yet be ruled out as an occasional route. In addition, an increasing number of farms are producing campylobacter-free flocks, often in succession (Anonymous, 2005), which also makes vertical transmission unlikely as a major route. Eggs have also not been identified as a source of human infection and the breeds of chickens used in commercial egg production are very different from those used for meat. In addition, unlike *S.* Enteritidis, campylobacters survive poorly in egg contents and on shells.

Control of campylobacter on the broiler farm

Chickens are no different from other food animals in that those reared under extensive (free-range) systems are more likely to be campylobacter-positive than housed animals (Heuer *et al.*, 2001). Given that campylobacters are commonly found in the natural environment it is not surprising that animals in direct contact with them are more likely to acquire the organisms. With outdoor-reared chickens challenge with campylobacters will be more frequent and of greater magnitude. There are currently no identified measures for the reliable control of this organism in this part of the industry, although it may be possible that dietary manipulation and/or phage treatment, which are discussed below, could be applicable to non-housed birds. This section will discuss control in housed chickens for the reasons discussed above and because, at present, these birds still represent the greatest share of the market in the developed world.

In its second report on campylobacters (Anonymous, 2005) the UK ACMSF stated that 'campylobacter control is possible for housed birds, as interventions in Scandinavia, particularly Norway and Sweden, have illustrated'. In these countries control is based on strict biosecurity and this approach is clearly successful, particularly in a country like Norway with very low flock infection rates of around 7% per annum. It has been argued that control is easier in Scandinavia because the industry is smaller and generally more profitable and because the winters are much harsher. This means that the environmental load of campylobacters is lower. It is important to determine whether control measures, which have been shown to be successful in Northern Europe, can be applied elsewhere, especially in Southern Europe where seasonal climatic differences are smaller. It is of interest that there is a marked increase in the number of broiler flocks that are campylobacter-positive in Norway during summer and these comprise >80% of the annual total of colonised flocks (Kruse, personal communication). The reasons for this have yet to be established but they could relate to higher environmental load and/or increased airflow through the houses. Before control can be properly applied it is important to identify the sources and routes of infection in housed flocks.

Prevention of horizontal transmission on-farm: This control measure is generally known by the term 'biosecurity' and is concerned with protecting birds from infection from outside sources. This is a very important control measure, because once campylobacters enter a poultry flock the spread can be rapid and currently impossible to control. Campylobacters enter the broiler house from the exterior environment, and the most important control measure is to prevent or, more realistically, to limit this entry. Thus, proper on-farm biosecurity is the major intervention measure for housed chickens. There is no doubt, however, that these bacteria are more difficult to exclude than salmonellas, as shown by the fact that there are many more salmonella-free flocks. Farmers and veterinarians will visit broiler flocks during the growth cycle and in many countries it is a welfare requirement that farmers visit their flocks each day. Each visit increases the infection risk and they should be limited to essential personnel. Hygiene is very important when entering flocks. The proper use of disinfectant foot dips can protect flocks from infection, but a better approach is to also use a hygiene barrier in the house area adjacent to the birds. Dedicated outer clothing and footwear should be present on the inside of the hygiene barrier and should be used by everyone entering the flock. Outside Scandinavia there is some scepticism about the long-term efficacy of this approach, but a study in the UK found that the adoption of 'Norwegian-like measures' reduced infection rates by 67%. Campylobacter infection rates were reduced by 50% if boot dips were changed frequently (Anonymous, 2005). The increased use of concrete and other cleanable areas would also improve farm hygiene because such surfaces are easier to keep clean and dry than grass or soil. General farm hygiene is also important and studies in many countries have shown that farms with poorer hygienic practices produced infected flocks. This could be linked to higher campylobacter loads in the environment.

Additional control measures

A range of additional control measures exist that could be applied to the control of campylobacters on broiler farms. These will not be discussed in detail here but are considered at greater length in the recently published ACMSF report mentioned earlier (Anonymous, 2005). The following could be applied to control infection on the farm:

1. **Competitive exclusion:** A healthy, balanced gut flora is vital for immune development in all animals and a combination of effective immunity and gut commensals will protect animals against challenge with enteric pathogens. Commercial chickens are generally reared under conditions which may not allow their gut flora to develop in a natural way. To overcome this the industry may use competitive exclusion (CE) with a cocktail of non-pathogenic gut bacteria for the control of salmonellae in poultry production. This approach has so far not been successful in the control of campylobacters. Commensal gut flora may be manipulated by changing the diet of the animal and some research has shown that chickens given certain diets are better able to resist challenge with campylobacters.

2. **Vaccination and the role of maternal antibodies:** Work is being undertaken in many countries to identify which campylobacter genes are important in the colonisation of the avian gut. This work could lead to vaccines against particular campylobacter cell targets. Chickens can mount an antibody response to challenge with campylobacters. In addition, high antibody levels have been found in breeder flocks and the yolks of their eggs (Sahin *et al.*, 2001). Chicks from such flocks can also be antibody-positive. However, data on the antibody responses to infection with campylobacters are equivocal and more work is needed on this before such a control measure can be adopted by industry. It is also important to ensure that the vaccine delivery is cost-effective.

3. **Treatment with bacteriophages:** Viruses known as bacteriophages (phages), which are found naturally in the chicken gut can attack campylobacters (Anonymous, 2005). This offers another potential control measure and it has been shown that the use of phage preparations can remove most of the campylobacter cells present in the chicken gut in the days immediately following treatment. It may thus be possible to treat a campylobacter-positive flock a few days before slaughter to either reduce or eliminate carriage of the bacteria. A possible limitation of this approach is that it might lead to an increase in phage-resistant campylobacter strains.

Flock management and campylobacter infection: Vaccination and drug therapy have been proposed as control measures although the use of antibiotics has resulted in the emergence of antibiotic-resistant campylobacter strains all over the world (Allos, 2001). This trend further emphasises the need for appropriate and safe use of antibiotics in animal production.

It is clear from data from some countries that farmers can differ in the frequency with which they produce campylobacter-positive housed broiler flocks. Other potential control measures may be identified by understanding why this is so, and there are many explanations as to why farms differ in campylobacter infection rates. This may just reflect differences in hygiene but there could be other reasons, and it is possible that birds in a poor environment are more susceptible to campylobacters as they will be to other pathogens like salmonellas. There are welfare and public health needs to identify why farming practices differ. If healthier and better managed chickens can resist campylobacters three potential benefits can be identified. Industry productivity and profitability will be improved, carcass contamination levels with campylobacters will be reduced and fewer people will be infected.

Campylobacters in other commercial poultry: As Table 3 shows, campylobacters can be found in other commercial poultry at frequencies similar to those in chicken. The control measures discussed above can also be applied to the production of these other species.

FOOD PROCESSING CONTROL MEASURES

*G*enerally speaking, foods are made safe through inactivation, e.g. heat treatment, and/or through inhibition of growth to prevent multiplication of pathogens to harmful levels. However, since campylobacters remain a concern even at low levels, their presence in foods at the point of consumption must be prevented, so growth inhibition is not relevant as a method of control. Compared to other pathogenic bacteria campylobacters are relatively heat sensitive so commercial heat processes set within a HACCP framework should guarantee control of these pathogens. We recognise the value of modelling pathogen behaviours in the food chain as a means of identifying the most effective and cost-effective control measures. Clearly, such an approach is possible with campylobacters. However, these pathogens are inherently variable and a large body of data, using many strains examined under a multiplicity of conditions, will be needed before modelling can be used with the necessary confidence.

Control during poultry processing: There is a high risk that campylobacters present on infected birds will be transmitted to other carcasses being processed e.g. during defeathering. This underscores the need to apply HACCP principles in processing plants to minimise product contamination. Critical control points and good manufacturing practices identified include temperature controls (washer and product), chemical interventions, water replacements, counter-flow technology in the scald tank and chiller, equipment maintenance, chlorinated-water sprays for equipment and working surfaces, increase in chlorine concentrations in process water and removal of unnecessary carcass contact surfaces (Mead *et al.*, 1995; White *et al.*, 1997). This issue is also discussed later in this review.

Table 4 gives details of the death rates of *C. jejuni* isolates under conditions relevant to food production. Data are taken from an International Commission on Microbiological Specifications for Foods (ICMSF) publication (Anonymous, 1996) and should be used as a guide only. Data do not take into account either strain-to-strain variation or the recovery and challenge methods used. Studies indicate that the apparent heat resistance observed when carcasses are scalded or pieces of poultry are heated (Figure 3) is not only relevant to campylobacters. For example, similar increases in apparent heat resistance are also observed with enterobacteriaceae. These (apparent) increases in resistance may be due to temperature deviation (e.g. the temperature of a carcass not reaching the temperature of the heating medium) or physical protection in high fat foods and there are studies describing these effects.

Table 4: Death rates in log$_{10}$ units of Campylobacter jejuni *in food production-relevant environment*

Environment	Temperature (°C)	Death rate (log$_{10}$)
Skim milk	- 20	-4.4 to -7.5 over 5 days
Ground chicken	- 18	-1.5 to -1.6 over 5 days
Raw milk	5	-2.8 over 2 days
Ground chicken	4	-0.5 to -2.0 over 7 days
Chicken scald water	52	-1.0 over 9 minutes
Ground beef	56	- 1.6 to -1.04 per minute
Lamb cubes	60	-5 to -3.8 per minute
Ground cod with 0.5% NaCl	10	- 1.6 over 5 days
Ground cod with 2.5% NaCl	10	- 2.8 over 5 days
Egg white at pH 9.3	42	- 7.1 over one day
Yogurt at pH 4.4-5.4	NS**	- 7.4 per hour
Ground turkey and 0.32 kGy	- 30	- 1.0

  * From Anonymous, 1996
  ** Not stated

Figure 3: Counts of campylobacters of broiler carcasses before and after various water treatments
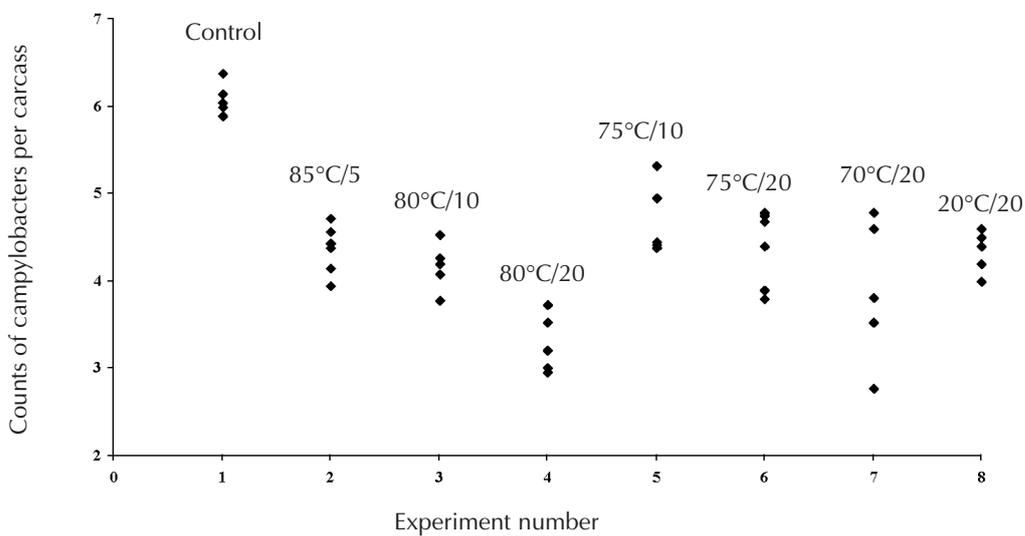
Counts are from a single 300 ml rinse plated on selective agar. Each point represents an individual carcass. Treatments are given as temperature of exposure in °C followed by the length of exposure in seconds. Data from Purnell *et al.* (2003).

Carcass decontamination methods other than heat: The importance of poultry as a source of human infection has led to studies on chemical carcass de-contamination at processing. Oyarzabal *et al.* (2004) found that the post-chill application of acidified sodium chlorite to chicken carcasses caused a significant reduction in campylobacter numbers and in the number of contaminated carcasses. Mead *et al.* (1995) examined chlorine use and saw a reduction in carcass campylobacter levels but they concluded that this was unlikely to be of public health significance. Chemical treatment of carcasses is not permitted in the EU but chlorine can be used in other countries such as the US and Brazil.

Rapid freezing of chicken carcasses may also offer additional control measures (Bhaduri and Cottrell, 2004; Sandberg *et al.*, 2005). Although data on the effects of this on the numbers and infectivity of campylobacters are lacking, data from Iceland suggest that frozen poultry poses a lower risk to health than fresh meat (Stern *et al.*, 2003).

Irradiation: Irradiation of food materials, using electron beams (from electron accelerators) or high-energy electromagnetic radiation (gamma-rays from ^{60}Co or X rays), is permitted in some European countries and will kill campylobacters and other infectious bacteria. The destruction rate depends on the type of food being processed and is less effective for frozen materials than those, which are chilled or at ambient temperature. Studies (Patterson, 1995; Clavero *et al.*, 1994) show that campylobacters are more susceptible to radiation than salmonellas and *Listeria monocytogenes*, with D values (the time taken to kill 90% of the population) varying between 0.12-0.25 kGy. Doses used to inactivate these pathogens will also kill campylobacters.

Factors affecting survival in food

Karenlampi and Hanninen (2004) showed that *C. jejuni* can survive on fresh produce long enough to pose a risk to the consumer. Survival times will depend on the food matrix involved and the conditions under which the foods are stored. However, data show that campylobacter numbers on chicken carcasses can remain largely unchanged for over seven days at refrigeration temperatures (Jørgensen *et al.*, 2002). Despite reported long-term survival, in almost all cases of human disease the infecting campylobacter population will have been exposed to hostile environments in particular high and/or low temperatures, before being consumed. It would seem clear that despite the reported sensitivity of this pathogen to the extra-intestinal environment, its infectious potential is not compromised by such exposures. Despite their importance as human pathogens little is known about how campylobacters cope with hostile conditions in the transmission chain from animals to humans and how these bacteria persist in foods or non-food environments. There is similarly scant information about the molecular mechanisms that enable campylobacter strains to survive environmental stress conditions relevant to food production. A myth has developed that thermophilic campylobacters are very sensitive to conditions outside the host. It is difficult to reconcile this view with the fact that these bacteria are able to infect approximately 1% of the population of Western Europe largely as a result of the consumption of contaminated food. There is no doubt that, when challenged in broth culture, campylobacters are more sensitive to heat and acid, for example, than salmonellas or *E. coli*. This may not reflect what happens in food as the data on the heat treatment of chickens (Figure 3) suggest. An additional problem is that the methods used in many of the published studies are not sufficiently sensitive to recover damaged cells and this may lead to an underestimation of resistance. We are on the threshold of learning more about campylobacters, particularly *C. jejuni*, than ever before. The genome sequence of *C. jejuni* strain 11168 was published a few years ago (Parkhill *et al.*, 2000). Sequencing and post-genomic studies are giving an insight into the metabolism of *C. jejuni* and how it might survive outside the host.

Gram-negative bacteria have regulators which mediate responses to environmental change. The most important is RpoS (Jørgensen *et al.*, 2000) which campylobacters do not have although they do have others (Park, 2002). Campylobacters can respond to changes in pH, temperature and available oxygen although the consequences that this has for food safety have yet to be examined. It is important to remember that campylobacters will not survive pasteurisation treatments or proper cooking. Campylobacters studied so far have shown high sensitivity to oxidative stress and have an inherent sensitivity towards oxygen and its reduction products (Humphrey, 1988). Oxidative stress plays a role in freeze-thaw killing of campylobacters and many isolation media have reduced sensitivity because they do not protect the bacteria from oxygen radicals generated during culture (Humphrey, 1988). Campylobacters are also particularly sensitive to osmotic shock and drying. This is likely to be because they lack systems for the synthesis or transport of compatible solutes. It has been reported that *C. jejuni* can mount a stringent stress response that is regulated by *SpoT* (Gaynor *et al.*, 2005). The 'stress' situations affected include stationary phase survival, growth and survival under low CO_2/high O_2 conditions and rifampicin resistance. The stringent stress response is also thought to be important for adherence, invasion and intracellular survival. A recent review (Murphy *et al.*, 2006) gives a comprehensive and authoritative overview of the environmental survival mechanisms of *C. jejuni* in more detail than can be covered in the present report.

Responses of *C. jejuni* to low temperatures: Cold shock proteins found in many other bacteria and typically associated with ability to grow at temperatures below the optimum for growth have not been found in *C. jejuni* (Park, 2002; Hazeleger *et al.*, 1998). This may, in part, explain why its minimum growth temperature is 30°C. Transitions in the structure of important enzyme(s) or regulatory compound(s) may also play a role (Hazeleger *et al.*, 1998). This does not mean that it is inactive at low temperature and cells are capable of prolonged survival under refrigeration. Low temperature is widely applied in the food chain, and it is vital that more become known about how this pathogen responds to these conditions with regard to survival and subsequent behaviour, as this has the greatest overall relevance to food safety. Low temperature is used at all stages of the poultry production process: carcasses are matured in the abattoir under low temperature regimes; whole chicken, chicken portions and cook-chill products are prepared and maintained under low temperature conditions; and the distribution and supply chain is, of course, maintained at low temperature. Very similar factors apply to other foods that can act as vehicles for infection by campylobacters such as raw cow's milk. Low temperatures are therefore central to the preparation, storage and distribution of food that may be contaminated with these organisms and a deeper understanding of how the bacteria respond to and survive in these conditions may help to inform interventions via the cold chain. Campylobacters, like other bacterial pathogens, can survive for extended periods at low temperature on key foods such as raw chicken and very high contamination levels have been reported. The means by which the bacteria do this are currently unknown.

Campylobacter jejuni cells show considerable metabolic activity, including *de novo* protein synthesis, chemotaxis and aerotaxis at low temperature, even as low as 4°C (Hazeleger *et al.*, 1998). There is also the very interesting observation that transient increases (around 0.5-1.0 $\log_{10}$) in colony counts were occasionally seen in a *C. jejuni* population held at 4°C (Ekweozor *et al.*, 1998). These authors ruled out experimental error and concluded that *C. jejuni* is capable of either growth at low temperatures or transition between temporary culturable or non-culturable states. Comparison of campylobacter strains revealed differences in survival profiles at low temperature, but the reasons for these are not known (Hazeleger *et al.*, 1998). In campylobacters the adaptive mechanisms underlying the responses to cold stress have not been examined in any detail although this is of considerable interest, given the involvement of chilled foods in infection. Campylobacters show marked changes in the fatty acid composition of chilled cells compared to those held at higher temperatures and there are differences in protein expression between cultures

held at either 4 or 20°C (Holler *et al.*, 1998). There is still much to learn about the ability of *C. jejuni* to survive low temperature. Data (Mattick *et al.*, 2003) show that *C. jejuni* responds to chill in a manner different from salmonellas and *E. coli*. Unlike these bacteria it does not become heat-sensitive when chilled. This may have important implications for food manufacturers and caterers, as the data demonstrate that campylobacters may be better able to survive certain processes than previously thought. It is important that food processors and caterers apply treatment regimes that take all relevant food-borne pathogens, their resistance to particular control measures and their probable maximum numbers into consideration.

Responses of *C. jejuni* to high temperature, low pH and desiccation: Data from laboratory studies demonstrate that *C. jejuni* is more sensitive to heat and acid than salmonellas, for example, in broth culture. Heat treatment regimens that destroy salmonellas are very likely to also kill campylobacters. However, it is too simplistic to regard *C. jejuni* as a highly sensitive pathogen, and it is important that food processors and caterers apply treatment regimes that are effective for all potential food-borne pathogens. In common with many other bacteria campylobacters are able to attach well to biological and other materials. One effect of this is to make attached cells much more heat tolerant than planktonic ones (Blankenship and Craven, 1982). This has also been seen with *E. coli* and coliforms (Berrang *et al.*, 2000). Other possible explanations for a reduced effect include a lower temperature at the surface of carcasses immersed in scalding tanks. The practical implications of attachment are still to be properly assessed. Studies in poultry processing plants have shown that the application of wet heat only had a marginal effect on the campylobacter contamination rates of carcasses (Berrang *et al.*, 2000; Purnell *et al.*, 2003). Figure 3 presents data on the treatment of naturally contaminated chicken carcasses with hot water at the end of the processing line before chilling and is adapted from the paper by Purnell *et al.* (2003). The study compared washing the carcasses with water at ambient temperature (20°C) to exposing them to water at high temperature. Somewhat surprisingly, the heat treatments had a much smaller effect than expected when compared to treatment with water at ambient temperature, even though in many cases the chicken skin was markedly changed and sufficiently damaged to render it very fragile. The good survival of the campylobacter cells is likely to have been a consequence of their attachment although there is the possibility of migration into the feather follicles. There is a need to examine properly the tolerance of attached campylobacter cells given that most people will become infected with such bacteria.

Although there is debate about the heat tolerance of campylobacters there is no doubt that these bacteria are much more sensitive to acid and drying than other food borne pathogens. Campylobacters survive poorly on dry surfaces, particularly in the absence of protective organic matter (Humphrey *et al.*, 1995). They are also killed by cleaning and disinfection regimes effective for other pathogens. *Campylobacter jejuni* mounts an acid tolerance response (Murphy *et al.*, 2003) but the practical implications of this have yet to be assessed.

Genetic variability: It appears that *C. jejuni* does not possess many of the adaptive responses present in other food borne pathogens but is an unusually diverse bacterial species as evidenced by significant strain-to-strain variability in virulence and tolerance to particular stresses (Park, 2005). This implies that strains can have important differences in their genetic makeup and this is confirmed by studies showing much more extensive differences in genetic content compared to other enteric bacteria. These differences are not confined to large rearrangements in DNA, and small changes are more common compared to other enteric pathogens (Park, 2005). Studies reviewed by Park (2005) illustrate *C. jejuni* diversity and highlight the presence of additional genetic material in some strains and, in turn, the potential for strain-specific mechanisms of stress tolerance.

Coccoid and non-culturable campylobacter: Campylobacter cells, and those of other bacterial foodborne pathogens, have been shown to be able to enter a Viable Non-Culturable (VNC) form (Rollins and Colwell, 1986). There have been many reports that *C. jejuni* changed from a spiral to a coccoid morphology and that this is associated with a loss of culturability although cells were still viable. There continues to be much debate over this, but it is clear that many of the techniques used to assess culturability were sub-optimal.

The commercial and public health significance of the work referred to above which is very similar to that published by Whyte *et al.* (2001), has yet to be evaluated. It does illustrate, however, that in a protective environment like chicken skin campylobacters are more difficult to remove than laboratory data suggest.

CAMPYLOBACTERS IN DOMESTIC AND COMMERCIAL KITCHENS

The World Health Organization (WHO) has outlined five keys to safer food. They are:

- Keep clean
- Separate raw and cooked
- Cook thoroughly
- Keep food at safe temperatures
- Use safe water and raw materials

This applies also to campylobacters and raw foods like poultry will continue to be sources of these in household kitchens and commercial catering. Such foods must therefore at all times be recognised as presenting a risk if not adequately cooked, or if they come into contact with ready-to-eat foods. It should also be borne in mind that the external packaging of poultry packaging, and occasionally that of red meat can be contaminated with campylobacters (Burgess *et al.*, 2005; Harrison *et al.*, 2001). Cross-contamination has been shown to be an important cause in approximately 30% of outbreaks and this can take many forms. The most important is direct contact between raw and cooked foods, but there are potentially more insidious forms of cross-contamination. Research has shown that meal preparation with heavily contaminated foods like raw chicken can lead to the widespread dissemination of campylobacters around the food preparation area (Cogan *et al.*, 1999). Thus, work surfaces, door and cupboard handles, chopping boards, dishcloths etc. can be positive for these pathogens, sometimes even after cleaning. The greater the degree of handling and portioning of carcasses the greater will be the spread of campylobacters. It is important that cleaning is applied to a wide area around the food preparation site. As recovery techniques for campylobacters improve it is becoming increasingly possible to demonstrate that the persistence of these bacteria, previously thought to be hypersensitive to the extra-intestinal environment, can be quite prolonged. It is possible, albeit infrequent, to isolate campylobacters from kitchen work surfaces 24 hours after raw chicken has been prepared (Cogan *et al.*, 1999). The plethora of kitchen hygiene products available for consumers to buy could suggest that a contaminated work surface or dishcloth poses a substantial risk. The circle has yet to be completed, however. Thus, while a substantial spread and degree of persistence has been demonstrated there has been no research to determine if the campylobacter cells present at the contaminated sites end up in foods to be consumed. What is needed, as part of a general risk assessment of the food chain, is an examination of surface contamination in both household and commercial food preparation areas and the risks that this poses to food safety and consumers.

In both household kitchens and commercial catering it is essential that raw foods contaminated with campylobacters be cooked properly. After cooking the food must be protected from re-contamination. There are many ways by which cooked food can be re-contaminated. As mentioned above campylobacters spread in kitchens during food preparation. An investigation into consumer food handling habits found that in > 50% of observations people did not wash their hands after handling raw meat/poultry. Given the high campylobacter contamination levels on chicken it is, perhaps, natural that consumers and caterers may wish to wash carcasses. Work showed that around 30% of consumers surveyed washed raw chicken. This will spread contaminated droplets. Fifteen percent did not always cook the food to a sufficiently high temperature.

As with other zoonotic pathogens contamination levels of campylobacters on kitchen surfaces can be effectively reduced by cleaning with detergents, hot water and disinfectants. However, consumers do not always do this correctly and it is possible to isolate campylobacters from kitchen surfaces even after 'cleaning' (Cogan *et al.*, 1999). Proper hand washing is also very important and a recent review paper stated that this practice could reduce the risk of diarrhoea in a community by ~ 50%.

DISCUSSION AND RESEARCH NEEDS

Discussion: This report has discussed extremely important zoonotic, food-borne pathogens, campylobacters, which worldwide infect millions of people each year. Infection, particularly in children, can be severe and in this group bloody diarrhoea is a common feature. Infection can also have debilitating long-term sequelae, causing substantial morbidity and placing a major cost burden on national health care systems. Most cases are caused by *C. jejuni* and this pathogen is unusual in that general outbreaks are only rarely identified, although that may change as typing and surveillance improve. A variety of infection vehicles has been identified, but there is general agreement that contaminated poultry meat is the most important. With that in mind this report concentrates on epidemiology and control of campylobacters in poultry production. Recent work has shown that with poultry that are housed control is possible by using strict hygiene measures to prevent horizontal transmission from the outside environment. While successful this control measure is not absolutely effective and other measures are required to reduce flock colonisation to the lowest levels possible. Vaccination and the use of bacteriophages are possibilities, but recent studies have demonstrated that improving flock health and welfare also may bring about reductions in the number of campylobacter-positive housed flocks. Control measures for birds reared in free-range systems present even greater challenges.

Campylobacters have long been regarded as very sensitive to the extra-intestinal environment and thus easier to kill than either salmonellas or *E. coli*. Whilst it would be wrong to regard campylobacters as highly resistant, it is becoming clear that they are more robust than previously thought. *Campylobacter jejuni* has been shown to be able to respond to a range of conditions relevant to food production and seems to have particularly novel responses to low temperature. This pathogen also appears to attach well to biological material and this can have a marked effect on heat tolerance under commercial conditions (Figure 3). Food producers should not panic as new data emerge but must not be seduced into thinking that campylobacters are easy to kill and relax treatment protocols accordingly. It is important that those involved in the production of food remember that although salmonellas and campylobacters are both Gram negative bacteria their behaviours are very different. Data generated on one pathogen will not necessarily be applicable to the other.

Certain foods, principally raw chicken meat, can have very high (> 10^7 cells per carcass) campylobacter contamination levels (Jørgensen *et al.*, 2002). These can lead to extensive cross-contamination in commercial and household food preparation areas. Cross-contamination has been shown to be an important infection risk factor. Control can be particularly difficult in the household environment and there is a need to identify the best ways to advise consumers on this subject. The post-genomic revolution means that more is now known about the gene expression in *C. jejuni* than ever before. It is important that this knowledge be used to the benefit of those who ultimately pay for the acquisition of the knowledge, namely the consumers.

Future research needs: There are many research areas which require further work. What follows are some ideas with relevance to the food industry. Many of them will also be relevant to risk assessment and to establish effective control measures at appropriate stages of the food chain.

- Determination of the reasons for different behaviours of campylobacters in humans and domestic animals.
- Examination of stress responses in campylobacters and the effects that these have on virulence and survival during food production.
- An examination of the mechanisms of campylobacter attachment and determination of the reasons for increased heat and acid resistance.
- A better understanding of transmission on-farm and an examination of the efficacy of on-farm control measures, which have been shown to be effective in one country or region, in other parts of the world.
- Determination of the driving forces for the frequent changes seen in the expression of surface antigens in campylobacters and the implications that these have for food safety.
- Examination of potential carcass treatments such as organic acids in industrial settings.
- Identification of the major risk factors for cross-contamination in commercial and domestic catering.
- Determination of optimum isolation/detection and typing methods.
- The relevance and importance of arcobacters as zoonotic pathogens.
- Improving understanding of the effects of rapid freezing.

CONSIDERATIONS FOR STAKEHOLDERS

Considerations for governments:

Public health authorities have an important role in reducing the prevalence of food borne campylobacteriosis by:

- Funding scientific research on campylobacters (e.g. to elucidate the relative significance of its transmission routes, virulence, epidemiology, consumer behaviour, etc).
- Improve data collection and reporting systems on campylobacteriosis and facilitate international collaboration and data exchange (which in turn requires harmonization of surveillance systems and detection and typing methods).
- Establish performance objectives for poultry farms and slaughter houses and implement monitoring programs to verify that these objectives are met.
- To examine, with stakeholders in the food chain, the economics of poultry production to ensure that control is not compromised by low profitability in primary production.
- Provide education to various stakeholders, in particular to farmers, on best practices for poultry production and to consumers on good hygienic practices with food that may be contaminated with campylobacters.
- The post-genomic revolution means that more is now known about the gene expression in *C. jejuni* than ever before. It is important that this knowledge be used to the benefit of those who ultimately pay for it, consumers.

Considerations for primary food production:

To reduce the prevalence of campylobacters in primary foods the emphasis should be on applying GAP and GHP principles. Of particular importance is the prevention of contact between contaminated sewage and animal farm waste and those primary foods that will not undergo heat processing at a later stage or that may become a source of cross contamination in the individual households.

Since poultry is considered to be the most important source of sporadic campylobacteriosis the following specific measures are recommended for poultry farms:

- Changing shoes or utilisation of a disinfecting shoe dip and washing hands before entering the houses.
- Providing clean drinking water to the animals.
- Applying appropriate pest control (birds, rodents and insects) and avoid contact with other animals such as cats, dogs, cows and pigs, either directly or indirectly through manure.
- Cleaning and disinfection of house and transport crates between one flock and the next.

Other specific measures for other primary foods that may be at risk from contamination with campylobacters include:

- Vegetables and fruit that are usually eaten raw must not be grown in areas where water used for irrigation may contain campylobacters nor should they be washed with water that do not meet drinking water quality requirements.
- To prevent contamination of raw milk GHP should be applied during milking.
- To prevent contamination of (drinking) water with campylobacters a reliable safety assurance system in water supply systems is essential.
- Prevention of contamination of bivalve molluscs can be achieved by controlling the quality of the water before harvesting and during depuration.

Considerations for abattoirs:

Campylobacters have to be considered as potential hazards in poultry and also, to a lesser extent, in other slaughter animals. Theoretically, it is possible to produce campylobacter-free meat by sourcing uninfected flocks and slaughtering the animals in a campylobacter-free environment, but this is still very difficult to achieve. Nevertheless, appropriate measures should be taken to reduce the prevalence of campylobacters such as:

- Strict adherence to GHP and GMP, in particular to prevent faecal contamination.
- After cleaning and disinfection the low risk animals (from campylobacter-free flocks) should be slaughtered first.
- Using appropriate physical (e.g. freezing, surface drying, surface steaming) or chemical decontamination techniques (where permitted).

To further reduce the risk for the consumer it is recommended that campylobacter-free flocks be channelled to retail fresh meat operations. Meat from infected flocks should preferably be used for production of processed foods. Alternative measures could include effective carcass decontamination and this should be explored.

Considerations for food processing industries:

In HACCP studies campylobacters have to be considered as potential hazards in raw meat, internal organs and offal from abattoirs, untreated water, raw milk, bivalve molluscs and fresh produce and adequate control measures should be defined. Examples include heat processing or irradiation, sourcing of fresh produce from suppliers that do not use contaminated water for irrigation or washing, etc.

Adherence to GMP and GHP is a prerequisite. An important element in this respect is the separation of raw ingredients from processed foods to prevent cross contamination.

Considerations for catering and food service:

Food handlers, including seasonal workers, need to be educated specifically about microbiological safety guidelines and hygiene rules. This should include training in hygienic measures to prevent cross contamination of ready-to-eat foods, in particular when handling raw poultry. To prevent undercooking adequate heating instructions and monitoring procedures should be available and implemented.

Considerations for retailers:

Retailers have a responsibility to provide consumers with adequate information about the microbiological hazards associated with the foods that are sold in their premises and also about basic hygiene rules on how to handle these food products at home.

Packaging materials used by the retailers should represent an adequate barrier against cross contamination.

Considerations for consumers:

Consumers need to be aware of the importance of respecting general hygiene rules when preparing food to avoid cross contamination, in particular when handling raw poultry.

It is recommended not to consume raw milk, undercooked poultry, or water from unknown or unprotected wells.

ABBREVIATIONS

ELISA	Enzyme-linked immuno-sorbent assay
GAP	Good agricultural practice
GBS	Guillain-Barré Syndrome
GHP	Good hygienic practice
GMP	Good manufacturing practice
ISO	International Organization for Standardization
MLST	Multi-locus sequence typing
PCR	Polymerase chain reaction
WHO	World Health Organization

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