SCIENTIFIC OPINION

Scientific Opinion on Quantification of the risk posed by broiler meat to human campylobacteriosis in the EU[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

This scientific opinion further elaborates a previous EFSA opinion and assesses the extent to which meat derived from broilers contributes to human campylobacteriosis at EU level. It gives an overview of the public health significance and burden of campylobacteriosis, concluding that there is considerable underascertainment and underreporting of clinical campylobacteriosis in the EU. The known and hypothesised factors having an impact of the epidemiology of human campylobacteriosis are summarised. Handling, preparation and consumption of broiler meat may account for 20% to 30% of human cases of campylobacteriosis, while 50% to 80% may be attributed to the chicken reservoir as a whole. Many factors may explain this difference in attribution. There are differences in the point of attribution (reservoir vs. point of consumption). Strains from the chicken reservoir may reach humans by pathways other than food (e.g. by the environment or by direct contact). Results may be biased by inaccurate exposure assessments, confounding by immunity and incomplete data on reservoirs. Data for source attribution in the EU are limited and unavailable for the majority of Member States and there are indications that the epidemiology of human campylobacteriosis differs between regions. Hence, the conclusions of this scientific opinion must be interpreted with care. Recommendations are made on EU surveillance and research activities aimed at improving quantification of the burden of campylobacteriosis, facilitating the evaluation of the human health effects of any interventions and giving a better basis for source attribution.

KEY WORDS

Broiler meat, chicken, campylobacteriosis, *Campylobacter coli*, *Campylobacter jejuni*, source attribution.

1 On request from the European Commission, Question No EFSA-Q-2008-469 adopted on 9 December 2009.

2 Panel members: Olivier Andreoletti, Herbert Budka, Sava Buncic, John D Collins, John Griffin, Tine Hald, Arie Hendrik Havelaar, James Hope, Günter Klein, James McLauchlin, Winy Messens, Christine Müller-Graf, Christophe Nguyen-The, Birgit Noerrung, Luisa Peixe, Miguel Prieto Maradona, Antonia Ricci, John Sofos, John Threlfall, Ivar Vågsholm, Emmanuel Vanopdenbosch. Correspondence: biohaz@efsa.europa.eu

3 Acknowledgement: The Panel wishes to thank the members of the Working Group on *Campylobacter* in broiler meat – Source attribution for the preparation of this opinion: Arie Hendrik Havelaar, Merete Hofshagen, Hilde Kruse, Noel McCarthy, Sara Monteiro Pires, Diane Newell, Norval Strachan.

Suggested citation: EFSA Panel on Biological Hazards (BIOHAZ); Scientific Opinion on Quantification of the risk posed by broiler meat to human campylobacteriosis in the EU. EFSA Journal 2010; 8(1):1437. [89 pp.]. doi:10.2903/j.efsa.2010.1437. Available online: www.efsa.europa.eu

SUMMARY

Following a request from the European Commission, the Panel on Biological Hazards (BIOHAZ Panel) was asked to deliver a scientific opinion on Quantification of the risk posed by broiler meat to human campylobacteriosis in the EU. In particular, the BIOHAZ Panel was requested to further elaborate a previous EFSA opinion and to assess the extent to which meat derived from broilers contributes to human campylobacteriosis at EU level. The importance may be expressed as a percentage of the total number of human campylobacteriosis cases.

The current scientific opinion gives an overview on the public health significance and burden of campylobacteriosis. There is considerable underascertainment and underreporting of campylobacteriosis and there may be not less than 2 million and possibly as high as 20 million cases of clinical campylobacteriosis per year in the EU27. The known and hypothesised factors that have an impact on the epidemiology of human campylobacteriosis are described (i.e. age, season, travel and food trade, strain variation, host immunity and demographic factors). Travelling outside the country of residence is a reported risk factor for human campylobacteriosis. A large proportion of cases is associated with travelling within the EU and would be preventable by EU-wide control measures.

The human illness source attribution methods currently applied are briefly summarised and the results obtained so far from the application of the different methods to the attribution of human campylobacteriosis cases are analysed. Handling, preparation and consumption of broiler meat may account for 20% to 30% of human cases of campylobacteriosis, while 50% to 80% may be attributed to the chicken reservoir as a whole.

Source attribution based on microbial subtyping has only recently been applied to campylobacteriosis. All subtyping techniques have shown the population structure of *C. jejuni* to be highly diverse and weakly clonal, with multiple subtypes having been described, many of which are rare. Some subtypes have a statistical association with specific reservoirs and there is considerable overlap between subtypes from cases of human disease and subtypes from farm animals, in particular from chicken. Most isolates for which subtyping data are available are not based on representative sampling. Current subtyping techniques do not provide any indication of strain virulence. Available studies from three geographical areas suggest that between 50 and 80% of all human cases of *C. jejuni* can be attributed to chickens as a reservoir.

A meta-analysis of case-control studies suggests a variety of risk factors including travelling, animal contact, food and untreated drinking water. Case-control studies also suggest that risk factors depend on the species of *Campylobacter*. It is noted that recent studies have estimated that between 24-29% of human cases can be attributed to handling, preparation and consumption of chicken meat.

Even though most cases of human campylobacteriosis are sporadic, outbreaks are reported in the EU. Recent source attribution studies based on outbreak investigations suggested that over one quarter of the outbreaks for which a source was identified can be attributed to chicken. For two thirds of the outbreaks the source was unknown.

"Natural experiments" in Belgium and the Netherlands in which the consumption of chicken meat was temporarily reduced supported the importance of chicken as a major reservoir of human infections, and chicken meat as an important pathway. Similarly, intervention studies in Iceland and New Zealand to reduce consumer exposure to highly contaminated chicken meat were accompanied by marked reductions in reported campylobacteriosis cases.

The lower proportion of human cases related to chicken meat identified by case-control studies as compared to microbial subtyping may be explained by several factors. There are differences in the point of attribution (reservoir vs. point of consumption). Strains from the chicken reservoir may reach humans by pathways other than food (e.g. by the environment or by direct contact). Results may be

biased by inaccurate exposure assessments, confounding by immunity and incomplete data on reservoirs.

Data for source attribution in the EU are limited and unavailable for the majority of Member States and there are indications that the epidemiology of human campylobacteriosis differs between regions. Hence, the BIOHAZ Panel states that the conclusions of this scientific opinion must be interpreted with care.

The BIOHAZ Panel recommends establishing active surveillance of campylobacteriosis in all Member States, including efforts to quantify the level of underascertainment and underreporting of the disease, in order to more precisely estimate the burden of the disease and allow the evaluation of the human health effects of any interventions. In order to provide a better understanding of the molecular epidemiology of campylobacteriosis and a better basis for source attribution, the BIOHAZ Panel also recommends that a representative collection of isolates from humans and putative reservoirs is obtained, subjected to genotyping in all Member States, and stored. Future research is also recommended in order to identify markers of *Campylobacter* virulence, survival properties and ecology and in order to quantify the impact of acquired protective immunity on the epidemiology of campylobacteriosis in the EU.

TABLE OF CONTENTS

Abstract 1
Summary 2
Table of contents 4
Background as provided by the European Commission 5
Terms of reference as provided by the European Commission 5
Assessment 7
1. Introduction 7
2. Public health significance of *Campylobacter* 8
2.1. Reported incidence of campylobacteriosis 8
2.2. Factors influencing campylobacteriosis epidemiology 10
2.2.1. Age 10
2.2.2. Season 11
2.2.3. Travel and food trade 12
2.2.4. Strain variation 13
2.2.5. Host immunity 13
2.2.6. Demographic factors 14
2.3. Burden of campylobacteriosis 14
2.3.1. Surveillance pyramid reconstruction 14
2.3.2. Sequelae and mortality 16
2.3.3. Antimicrobial resistance 17
3. Human illness source attribution methods 17
4. Attribution through microbiological approaches 18
4.1. Microbial subtyping 18
4.1.1. Methodology 18
4.1.2. *Campylobacter* attribution using subtyping 19
4.1.3. Implementation of *Campylobacter* subtyping methods in the EU 21
4.1.4. Modelling attribution approaches based on microbial subtyping 22
4.1.4.1. The Dutch Model 22
4.1.4.2. Population STRUCTURE 22
4.1.4.3. Hald Model 23
4.1.4.4. Modified Hald Model 23
4.1.4.5. Asymmetric island (AI) model 23
4.1.4.6. Self attribution in source attribution modelling 24
4.2. Comparative exposure assessment 27
5. Attribution through epidemiological approaches 27
5.1. Analysis of sporadic cases 27
5.2. Analysis of data from outbreak investigations 30
6. Attribution through intervention studies 34
7. Attribution through expert elicitation 36
8. Discussion 37
8.1. Chicken and broiler meat as sources of human campylobacteriosis 37
8.2. Strengths and weaknesses of attribution methods 37
8.3. Data quality and availability 42
Conclusions and recommendations 43
References 45
Appendices 56
A. Microbial subtyping methods 56
B. Modelling attribution approaches based on microbial subtyping 78
C. Methodologies for source attribution of human campylobacteriosis by epidemiological approaches 82
Glossary 88

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

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

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

In accordance with Commission Decision 2007/516/EC[5], all Member States are currently carrying out a harmonised baseline survey on the prevalence and antimicrobial resistance of *Campylobacter* spp. in broiler flocks and broiler carcases. On carcases, both qualitative and quantitative analyses are performed. The survey will provide reference values, comparable between Member States, in order to consider future performance objectives/targets along the broiler meat production chain. The results will be forwarded to EFSA by the end of April 2009.

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

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

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

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

The Commission approach and collaboration between risk managers and risk assessors in this area is indicated in the flow chart in the Appendix which was also forwarded to you in my letter (Ref 520106) of 2 April 2008.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

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

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

(1) Assess the extent to which meat derived from broilers contributes to human campylobacteriosis at EU level. The importance may be expressed as a percentage of the total number of human campylobacteriosis cases.

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

The deadline for submission is December 2009.

ASSESSMENT

1. Introduction

Campylobacteriosis is an infectious disease caused by members of a group of Gram negative curved or S-shaped motile bacteria. These bacteria were initially recognised at the beginning of the 20th Century as veterinary pathogens causing infectious abortion in cattle and were called "Vibrios" (McFadyean and Stockman, 1913; Smith and Taylor, 1919). Several decades later, King (1957) recognised the similarity of these pathogens to "related-vibrios" or "vibro-like" organisms from the blood samples of humans with enteritis and highlighted the need for improved diagnostic methods (King, 1962). Such methods were eventually developed by the end of the 1970s (Dekeyser et al., 1972; Skirrow, 1977) enabling the importance of this disease to become established by 1981 when the First International Workshop on *Campylobacter* was organized and by which time the zoonotic nature of the disease was being recognized (Newell, 1982).

The genus *Campylobacter* now comprises 17 member species most of which are microaerophils, i.e. grow preferentially in low oxygen concentrations. The majority of cases of campylobacteriosis are caused by two species: *Campylobacter jejuni* and the closely-related *C. coli*. These two species are often referred to as the "thermophilic" or "thermotolerant" *Campylobacter* as they grow preferentially at 42°C. When combined with selective media, the fastidious nature of these organisms can be exploited to ensure isolation and culture from the complex gut flora in faecal material. Other *Campylobacter* species, such as *C. fetus*, can also cause enteric disease but are relatively rare and usually would not be detected using routine culture methods. This opinion will focus on the species *C. jejuni* and *C. coli* as the major causes of the disease.

C. jejuni and *C. coli* are common components of the gut flora of all warm-blooded animals including livestock (cattle, sheep and pigs), domestic pets and wild animals but for both species the avian gut, with a temperature of 42°C, appears to be a preferential niche. Unfortunately during routine surveillance not all *Campylobacter* isolates are speciated. Nevertheless some interesting differences in the distribution of *C. jejuni* and *C. coli* in the various host reservoirs have been reported. For example, EU data reported for 2007 indicates that *C. jejuni* was identified in 42.5%, 86.8% and 2.1% of the isolates from broilers, cattle and pigs respectively, while *C. coli* was identified in 13.1%, 9.5% and 87.1% of the isolates from the same animal species (EFSA, 2009b). Few other *Campylobacter* species were identified in such isolates, however approximately 46.4%, 3.1% and 10.7% of isolates in broilers, cattle and pigs were reported as "unspecified" *Campylobacter* species. The proportion of unspeciated isolates varied between Member States (MS) as well as animal species. Some data are also available from national surveys. For example, in Great Britain, in a national survey of cattle, sheep and pigs at slaughter *C. coli* was identified in 9%, 34% and 90% of *Campylobacter* isolates respectively while *C. jejuni* was identified in 81%, 65% and 4% respectively (Milnes et al., 2008). In comparison, in a similar national survey in Great Britain of *Campylobacter* in poultry flocks at slaughter 77% were *C. jejuni* and 23% *C. coli* (Powell et al., 2009), while the corresponding results in Norway were 91% *C. jejuni*, 7% *C. coli* and 2% *C. lari* (Hofshagen and Kruse, 2005). These results suggest preferential and differential association between these *Campylobacter* species and livestock hosts. The bases of such ecological differences are as yet unknown.

In the vast majority of cases, the organisms are constantly shed in faeces by asymptomatic animals. Although campylobacters do not grow outside the host gut, they have the ability to survive for considerable periods especially in conditions that are moist, cool and out of direct sunlight. Consequently *C. jejuni* and *C. coli* are ubiquitous in the environment. Therefore there are many host reservoirs in which the organism is amplified and many vehicles by which the organism can reach its next host.

Campylobacter may be transmitted from these reservoirs to humans by many different routes. They can be transported directly from animals (i.e. skin contaminated with faeces) to humans by hands etc. (for example, by petting the animal and then subsequently using the hands to touch food or mouth directly). In addition *Campylobacter* are shed into the environment by live animals and can then be ingested by humans, for example, by drinking untreated water, through recreational contact with surface water or by eating contaminated raw vegetables. Other indirect routes have been hypothesised. For example *Campylobacter* in the environment might reach humans *via* vectors, such as flies, which might deposit the bacteria onto food (Nichols, 2005).

Meat also has an important direct and indirect role in the transmission of *Campylobacter*. The intestinal contents of livestock often contaminate carcases at slaughter. This is especially true for poultry, where the nature of the slaughter process enables more extensive faecal contamination than in mammals. If the animal is colonised with *Campylobacter* and a contamination of the carcase occurs, then these organisms will be distributed over the carcase surface and survive under the moist, cool storage conditions through to retail. Because of the processing and storage methods used, red meats (beef, lamb and pork) are less likely to be contaminated than poultry meat. *Campylobacter* can also be recovered from the deep muscle of poultry, albeit at a lower prevalence (20% vs. 87%) and concentration (mean 0.24 CFU/g vs. mean 537 CFU/g) than surface recovery (Luber and Bartelt, 2007). Such observations may reflect bacteraemic infection or result from the ingress of organisms into the tissue during processing for example *via* the lungs or feather sockets. Little information is available on extraintestinal colonization in other livestock but campylobacters are often recovered from pig's liver at retail. Meat contaminated by *Campylobacter* can either infect humans directly (if not properly cooked or otherwise treated) or indirectly. For indirect contact the contaminated meat acts as a vehicle of campylobacters, especially those present in meat juices, which can easily contaminate kitchen equipment such as cutting boards, plates, knives etc. and thereby other foods (salads etc.), which might be eaten without further bacteriocidal treatment.

The efficacy of the different transmission routes to humans, and their relative importance, will depend on a variety of factors including the number of campylobacters per exposure, the colonization and virulence potential of those campylobacters and host factors such as immunity and age. These factors will be discussed within Section 2 of the opinion.

A previous EFSA opinion (EFSA, 2005b) concluded that poultry meat appeared to be a major source of sporadic campylobacteriosis. Other food categories identified as potential sources included barbecued foods, untreated drinking water, undercooked pork, and poorly-pasteurised or raw milk. It was concluded that there was a need for a better understanding of their relative contribution to the incidence of campylobacteriosis and a quantitative analysis was not undertaken.

This opinion will describe the public health significance of *Campylobacter* infection in the EU, and will specifically aim to quantify the relative contributions of different reservoirs and vehicles (see definitions in Glossary) to human infection, with a special emphasis on broiler meat.

2. Public health significance of *Campylobacter*

2.1. Reported incidence of campylobacteriosis

In immunologically-naive humans, the symptoms of *Campylobacter* infection can range from mild, watery diarrhoea to severe bloody diarrhoea with fever, abdominal cramps and an influx of polymorphonuclear leucocytes (dysentery-like disease) (van Vliet and Ketley, 2001). Acute infections may also rarely involve intestinal complications such as appendicitis and colitis, and extra-intestinal infections, such as bacteraemia, hepatitis, abortion/perinatal infection and haemolytic-uremic syndrome (Skirrow and Blaser, 2000).

In 2007, more than 200,000 confirmed cases of human campylobacteriosis were reported by 24 MS, with an EU notification rate of 45.2 cases per 100,000 inhabitants. Notification rates for 2007 for the different EU MS are reported in Table 1. The variation in notification rates among MS is large (from 0.5 to 234.6) but the different sensitivities of the reporting systems and microbiological methods employed by MS will have influenced these figures. As a consequence, comparisons between countries should be carried out with extreme caution. This is further discussed in Section 2.3.1 of the opinion.

Table 1: Campylobacteriosis notification rates (based on reported cases) in humans (per 100,000 population) in the EU in 2007 (source EFSA (2009b)).

MS	Rate	MS	Rate	MS	Rate
Austria	70.1	Greece	n.r.	Portugal	n.r.[1]
Belgium	55.8	Hungary	57.7	Romania	n.r.[1]
Bulgaria	0.5	Ireland	43.7	Slovakia	62.7
Cyprus	2.2	Italy	1.1	Slovenia	56.1
Czech Republic	234.6	Latvia	n.r.	Spain	11.4
Denmark	71.0	Lithuania	16.7	Sweden	78.0
Estonia	8.5	Luxembourg	72.5	United Kingdom	95.0
Finland	77.8	Malta	22.3		
France	4.8	Netherlands	38.6		
Germany	80.3	Poland	0.5		

n.r. = not reported
[1] = no surveillance system in place

Data available for 2007 show an increase of 14.2% (almost 25,000 cases) in reported cases compared to 2006 (EFSA, 2009b). However, the EU notification rate decreased over the same period from 47.1 to 45.2 per 100,000 inhabitants. This is partly explained by the entry into the EU of two new MS, both with large populations and low numbers of reported campylobacteriosis cases. With the exception of Estonia, Hungary, Lithuania, the Netherlands and Spain, all MS reported an increase in the number of confirmed cases in 2007, compared to 2006, with the largest increases reported in Germany (27.0%) and the UK (10.9%). The decrease in reported cases between 2005 and 2006 is mainly due to the decrease in reported cases in two MS (Czech Republic and Germany).

When comparing data at the EU level it should be borne in mind that figures can change due, for instance, to a variance in surveillance and reporting systems or in diagnostic methods used in one or more MS. Such changes would not necessarily reflect an overall changing trend throughout the EU. To facilitate comparisons over different years, Figure 1 indicates the number of campylobacteriosis cases for nine EU MS that consistently reported during the period 1999-2007.

In five European countries (Iceland, Norway, Sweden, Denmark and the Netherlands), the long term de-seasonalised trend for domestic campylobacteriosis during the period 1997-2007 has been studied. For Denmark, Sweden and Iceland, there was a steadily decreasing trend in the period from 1999(Iceland) - 2001(Denmark/Sweden) to 2007, but for Norway the trend was steadily increasing throughout the period and in the Netherlands there was no obvious trend (Jore et al., 2009). In England and Wales reported cases of campylobacteriosis in humans showed an increasing trend from 1989 to 2000 when there were over 58,000 cases. The incidence then decreased until 2006 (at almost 47,000 cases) and increased again in 2007 (at almost 52,000 cases)[9].

[9] Source: Health Protection Agency website, figures reported from diagnostic laboratories to the HPA's local and national surveillance.

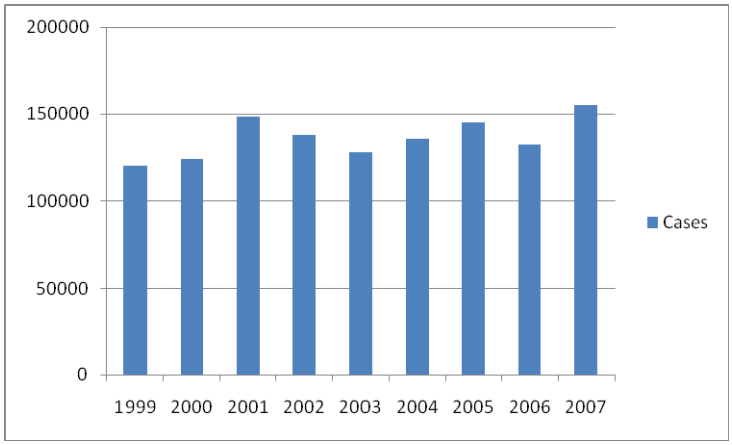

Figure 1: Campylobacteriosis cases in nine EU MS consistently reporting in the period 1999-2007 (Austria, Denmark, Finland, Germany, Ireland, Spain, Sweden, the Netherlands and the United Kingdom).

In the EU, reported food-borne outbreaks of campylobacteriosis are rare and outbreak-related cases represented around 2% of the total reported campylobacteriosis cases in 2004 and around 1% in 2005 and 2006 (EFSA, 2005a, 2006, 2007). In 2007 cases linked to verified outbreaks represented only 0.12% of the total reported campylobacteriosis cases, but this figure is not comparable with previous years due to a recent change in the reporting system (EFSA, 2009a). Because the identification of minor outbreaks is difficult, these figures may under-report the true incidence. For example, a number of sporadic cases of campylobacteriosis might actually represent unidentified household outbreaks. Household outbreaks are the most common type of *Campylobacter* outbreaks and comprise 3-5% of infections in Denmark (Ethelberg et al., 2004) and in Wales (Ribeiro and Frost, 2000). In two-thirds of household-associated outbreaks in one region of south Wales (Ribeiro and Frost, 2000) the patients involved were infected with an identical strain, as defined by sero- and phage-typing. Food vehicles, such as chicken liver pate (Forbes et al., 2009) may be contaminated with several strains, resulting in multiple-strain outbreaks, which would hinder the identification of individual outbreaks. Further information on outbreaks is given in Section 5.2 of this opinion.

Data related to *Campylobacter* species distribution in human campylobacteriosis cases are limited. Most laboratories do not routinely speciate clinical *Campylobacter* isolates, nevertheless, expert opinion worldwide indicates that about 80-90% of isolates are *C. jejuni* and 5-10% *C. coli* (Nachamkin et al., 2000). In 2007 in the EU, the *Campylobacter* species most frequently identified in isolates was *C. jejuni* (44.3%) followed by *C. coli* (2.7%); other *Campylobacter* species were aggregated and represented 6.9% of isolates. The remaining isolates (46%) were unspeciated (EFSA, 2009b). Overall data collected in the two previous years were similar (EFSA, 2006, 2007), however, there were significant variations between MS in the proportion of human isolates speciated.

2.2. Factors influencing campylobacteriosis epidemiology

Several factors are known, or are hypothesized, to have an impact on the epidemiology of human campylobacteriosis. The main factors are briefly reported below.

2.2.1. Age

Within the whole EU, in 2007, children under the age of 5 had the highest notification rate, representing 120 campylobacteriosis cases per population of 100,000. The rates for other age groups varied between 32 (age group 45-64 years) and 53 (age group 15-24 years) cases per 100,000

population. Data reported for 2007 were similar to those reported for 2006, 2005 and 2004 (EFSA, 2005a, 2006, 2007, 2009b).

Country-specific information on trends within the EU is scarce. However available data from England and Wales from 1990 to 2007 (Gillespie et al., 2009) indicate that the proportion of cases reported for patients of different age groups has slowly changed with a general decline in campylobacteriosis in young children under the age of 9 years, while older groups, and in particular the over 60 year olds, have seen an increase in infection over the last 10 years (see Figure 2).

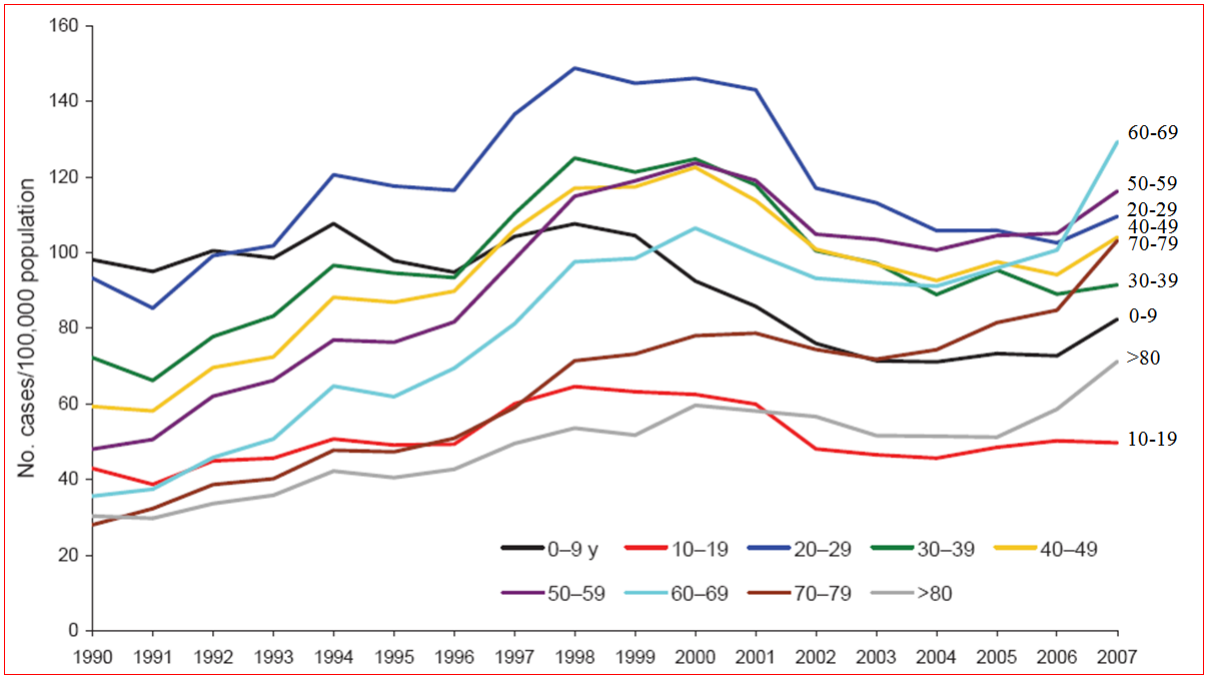

Figure 2: Incidence of laboratory-reported campylobacteriosis, England and Wales, by age group, 1990-2007 (Gillespie et al., 2009).

2.2.2. Season

Season correlates with the number of reported campylobacteriosis cases to a variable extent depending on the geographical area. According to EU-wide data in 2007 a higher number of campylobacteriosis cases were reported during the summer months (from June to August) (EFSA, 2009b). Similar observations were made in previous years.

In Northern Europe (Iceland, Norway, Sweden, Finland, Denmark and the Netherlands), a seasonal summer peak is consistently seen (peak month July or August) for domestic human cases. The peak is most pronounced for Finland, followed by Iceland and Denmark, while the Netherlands has the least pronounced peak (data from 1990-2007) (Jore et al., 2009). A previous study (Nylen et al., 2002), also reported similar peaks in late spring or summer in several northern European countries, as well as in New Zealand. Based on data collected over several years during the 1990s, Wales had the earliest peak of positive human cases occurring at the end of May, followed three weeks later by Scotland and five to seven weeks later by the Scandinavian countries (Finland, Denmark, Sweden). In New Zealand the pattern was different, with the peak period more variable from year to year and a more prolonged summer increase.

In a comparative study (Kovats et al., 2005) of southern European countries, a seasonal peak in campylobacteriosis cases was barely seen (Spain), or was more apparent in spring than summer

(Greece, Malta), though summer peaks occurred to a greater extent in Scotland, England and Wales, Ireland, the Czech Republic, Switzerland, Denmark and The Netherlands.

A recent study in the Netherlands investigating regional and seasonal differences in the incidence and antimicrobial resistance of *Campylobacter* (van Hees et al., 2007) indicated an inverse relationship between the seasonal campylobacteriosis summer peak and the levels of fluoroquinolone and macrolide resistance in *Campylobacter*. One possible explanation is that different sources of *Campylobacter* (e.g. swimming water) predominate during summer, and are associated with lower rates of antimicrobial resistance.

2.2.3. Travel and food trade

The origin of zoonosis cases reported in MS (i.e. cases acquired domestically versus cases acquired abroad) is indicated in the annual EFSA/ECDC Community Summary Report. In 2007, 61.6% of confirmed campylobacteriosis cases were reported as domestic, 6.8% as imported and the remainder (31.6%) as of unknown origin. Imported cases in 2007 decreased compared to 2006 (8.5%) and 2005 (8.0%) and fewer cases were reported as of unknown origin. The association between travel and campylobacteriosis is unknown for the majority of cases in 8 MS and 1 non-MS[10] (EFSA, 2009b). Because the number of MS reporting such data has varied over the years (i.e. 13 MS in 2005, 21 in 2006 and 23 in 2007) then these trends may not reflect the real situation (EFSA, 2006, 2007, 2009b).

The ratio of domestically acquired to imported cases varies depending on the country. In 2007, Nordic countries reported a high proportion of cases associated with foreign travel (Sweden 65.3%, Finland 56.6% and Norway 51.0%) while this proportion is less than 10% in most MS in other regions.

A detailed study in Sweden (1997-2003) examined specific risk factors for 28,704 travel-associated cases of campylobacteriosis, which represented 54% of all notified cases (Ekdahl and Andersson, 2004). Relative risks were highest for travellers to the Indian subcontinent, and other parts of Asia, and to Africa. The highest absolute numbers of cases were associated with travel to Thailand, Spain and Turkey. In total, 10,988 cases (38%) were associated with travel within Europe, which seemed to be the main travelling destination of Swedish travellers, and an additional 3,260 with travel to the Eastern Mediterranean. Hence, up to 50% of all travel-associated cases of campylobacteriosis in Sweden were related to travel within the EU.

In the UK, as part of a sentinel surveillance project, linked microbiological and epidemiological data were obtained from 7,360 patients infected with *C. jejuni*. 1,444 of these cases (20%) reported travel outside the UK in the two weeks before the onset of illness (Charlett et al., 2003). Travel in most EU countries had the same relative risk as travel in the UK but travel to Africa, Asia, the Pacific (other than New Zealand) and central/south America had a very high risk (over 10 fold) of campylobacteriosis. It is notable that travel to New Zealand only constituted a 3 fold increased risk of disease. In a more recent case-control study 24% of cases travelled abroad in the previous 14 days compared with 11% of controls (Tam et al., 2009).

Antimicrobial resistance patterns may also provide some insight into the impact of travel and the food trade on the exposure to *Campylobacter*. In countries with a very low usage of fluoroquinolones in poultry (Denmark and Norway) studies have shown a low proportion of fluoroquinolone-resistant *Campylobacter* among domestically-acquired campylobacteriosis cases and among domestic poultry. In comparison, travel-related campylobacteriosis cases had a high proportion of fluoroquinolone resistant strains (Charlett et al., 2002; Helms et al., 2005; Norstrom et al., 2006). These findings might also indicate that in these countries domestic cases are mainly caused by domestically-produced food

[10] Belgium, Bulgaria, Denmark, France, Ireland, Italy, Slovenia, the United Kingdom and Liechtenstein.

or other domestic sources and that imported food or patients returning from abroad play a minor role as sources for *Campylobacter* infection.

In conclusion, although many MS report travel-related cases separately from domestic cases, reporting differs between MS and the proportion of travel-related cases varies between countries. Travel is overestimated as risk factor, in particular due to the fact that no correction is made for observations in healthy controls. Furthermore, a large proportion of such cases is related with travel within the EU, and would be preventable by EU-wide control measures.

2.2.4. Strain variation

The mechanisms by which *C. jejuni* and *C. coli* cause disease symptoms in humans are unknown. There is no obvious difference in the clinical presentations by *C. jejuni* or *C. coli*, suggesting that mechanisms of pathogenicity are common to both species. The invasive potential of *C. jejuni* and *C. coli* is well-recognised from *in vitro* and *in vivo* experimental models. Invasion would account for the rare extra-intestinal infections, including bacteraemia and abortion, as well as the dysentery-like symptoms experienced by some patients. Invasiveness can vary between strains (Fearnley et al., 2008) but in general the invasiveness of campylobacters *in vitro* is poor compared with other enteric invasive bacteria. Nevertheless, several potential virulence genes associated with the adherence to and invasion of mammalian cells have been reported. The role of toxins in campylobacteriosis is undetermined (Wassenaar, 1997). Certainly the watery-like diarrhoea experienced by many patients is consistent with enterotoxin expression and several toxin-like activities have been described in *Campylobacter*. The genome sequences of these organisms have only identified the cytolethal distending toxin (CDT) as a known potential virulence factor in *C. jejuni* or *C. coli*. The *cdtA*, *B* and *C* genes are highly conserved in *C. jejuni* but have variable active expression, and even no expression, as a result of multiple naturally-occuring mutations (Abuoun et al., 2005). Consequently the expression of CDT has no obvious association with clinical disease and its importance in virulence remains unknown. Thus it seems that the classical mechanisms of bacterially-mediated diarrhoea are not clearly defined in *Campylobacter*. Several alternative mechanisms have been suggested. In particular the stimulation of host immmunopathological events *via* proinflammatory cytokine expression may result in disruption of the integrity of the intestinal mucosa and cause fluid accumulation (van Vliet and Ketley, 2001).

The major hindrance to identification of the virulence mechanisms of campylobacters is the absence of an appropriate animal model of disease (Newell, 2001). Most laboratory model animals challenged with campylobacters become temporarily, but asymptomatically, colonised. More exotic models using non-human primates, gnotobiotic or specific-pathogen-free (SPF) piglets or even ferrets are reported to become diseased but these models may demonstrate variable symptoms, poor reproducibility in other laboratories or be ethically unacceptable (Newell, 2001). Certainly such models would be unsuitable for screening large numbers of mutants to detect defined virulence factors.

Despite the lack of firm evidence, the huge genetic diversity within both *C. jejuni* and *C. coli* populations, which will be discussed later (see Section 4.1), strongly indicates that not all strains can be considered equally pathogenic. Moreover, the physiological status of the organism at the time of human exposure, i.e. whether it was environmentally stressed by factors such as atmospheric oxygen, dehydration or nutrient deprivation, may also have an effect on virulence potential. If certain strains are less pathogenic than others then this could potentially influence epidemiological and source attribution studies.

2.2.5. Host immunity

Individuals exposed to and colonized by *Campylobacter* develop both systemic and cellular specific immune responses. These responses may confer protective immunity from subsequent exposure and

enhance resistance to colonization and/or disease. Acquired immunity is generally accepted to be an important factor in the epidemiology of campylobacteriosis. In the developing world symptomatic infection is primarily restricted to children under two years of age. In the industrialized world, children under five years of age also have the highest incidence of disease but healthy adults can also develop campylobacteriosis. This is interpreted that regular exposure can confer protective immunity. Serosurveillance suggests that even in developed countries the majority of *Campylobacter* infections are asymptomatic, consistent with acquired immunity. In epidemiological studies on campylobacteriosis, immune status has not been taken into account which could lead to the misclassification of healthy persons as unexposed, and in risk assessment studies, health risks may be overestimated if immunity has been ignored (Havelaar et al., 2009).

In addition to acquired immunity, innate immunity may also affect susceptibility to infection and disease. This would be a consequence of host genetic complement and largely independent of previous exposure. Our knowledge in this area is poor; such factors have been recently reviewed (Doorduyn et al., 2008; Havelaar et al., 2009; Janssen et al., 2008).

2.2.6. Demographic factors

The influence of demographic factors (other than age) on the exposure patterns and hence the risk of human campylobacteriosis is unclear but various studies have indicated that ethnicity, urban/rural location and socio-economic status may all affect the epidemiology of this disease.

In the UK sentinel surveillance project (Gillespie, 2003) ethnicity was a specific risk factor for *Campylobacter* infection. The Pakistani community was at greater risk (almost 2 fold) of *Campylobacter* infection than the White community. In contrast the Indian and Black communities were at lower risk than the White community, while the risk in the Chinese community was no different from the other ethnic groups. Epidemiological differences between Pakistani and White cases were identified; in particular young Pakistani infants displayed a developing world epidemiology even though they had not travelled abroad.

It has been demonstrated that in the Grampian region of Scotland (Strachan et al., 2009) children (<5 years old) are 1.6 times more likely to present with campylobacteriosis than their urban counterparts and similar results have been obtained in a registry based population study in Denmark (Ethelberg et al., 2005). However, urban-rural differences for adult populations were not observed. A recent study in New Zealand (Mullner, 2009) also found that, particularly in the case of young children, cases in urban areas were more likely to be infected with poultry-associated sequence types (STs), while cases in rural areas were more likely to be infected with ruminant-associated STs.

Campylobacter has also been reported to have higher incidence in wealthy populations (e.g. in Denmark (Simonsen et al., 2008)) and the converse is true for deprived groups (e.g. in Gloucestershire (O'Neill et al., 2004)). It is unclear whether this difference is due to variation in reporting rates or differences in exposure to *Campylobacter* between different socioeconomic groups.

2.3. Burden of campylobacteriosis

2.3.1. Surveillance pyramid reconstruction

As previously addressed by EFSA in the scientific opinions on "A quantitative microbiological risk assessment on *Salmonella* in meat: Source attribution for human salmonellosis from meat" (EFSA, 2008b) and on "Quantitative estimation of the impact of setting a new target for the reduction of *Salmonella* in breeding hens of *Gallus gallus*" (EFSA, 2009f), most ongoing surveillance schemes for food-borne disease depend upon symptomatic patients consulting with, or presenting to, a primary care physician. Without this step the illness is unlikely to be recorded in any official statistics. The

loss of data at various points along the surveillance chain from patient, through laboratory tests, to official statistics is generally described as a pyramid. Disease in the community forms the base of the pyramid while those cases that reach official statistics form the apex. Surveillance systems "eavesdrop" on the healthcare system, and their organisation in MS varies considerably. For example, the surveillance system in the UK is highly centralised whilst those in MS like Germany and Spain are highly federalised. How differences in the organisation of surveillance might impact on reporting efficiency has not been investigated in a systematic way across the EU. It is likely that there is considerable variation in reporting efficiency across MS.

There have been relatively few attempts to calibrate *Campylobacter* surveillance data at national surveillance institutes, but some researchers have attempted to equate disease in the population to the data that appears in official statistics. In a three year study of infectious intestinal disease (IID) in the UK in the mid 1990s the investigators determined that for every laboratory-confirmed case of campylobacteriosis reported to national surveillance, 7.6 cases occurred in the community (Wheeler et al., 1999). This means that national statistics on laboratory-confirmed cases in England need to be multiplied by 7.6 in order to more accurately describe the community burden of campylobacteriosis. Since the mid 1990s there have been several changes in the surveillance of IID in the UK. The result of these changes is unknown. The IID study is currently being repeated in the UK and it will be interesting to determine if changes in the reporting pyramid are observed.

There are few similar examples from other EU countries on community-based studies. ECDC has recently launched a "call for tender" on a literature review of the factors affecting comparability of food-and waterborne disease incidence rate estimates based on notification data.

In Australia, the reported rate of campylobacteriosis is approximately nine-fold higher than in the USA. This difference cannot be explained by variations in the respective surveillance pyramids (Vally et al., 2009). Moreover the adjusted incidence rates indicate that reported rate should actually be twelve times higher in Australia compared to the USA.

Recent seroepidemiological studies suggest that many people have circulating antibodies to *Campylobacter* without obvious disease. This has been interpreted to indicate that a large proportion of the population are exposed to the organism, and may be colonized, without becoming ill. Havelaar et al. (2009) estimate that in the UK and in the Netherlands, only 1 out of approximately 100 infections leads to clinical illness. Ang et al. (2007) demonstrated an increasing incidence of sero-prevalence for anti-*Campylobacter* IgG antibodies in a representative sample of the Dutch population (De Melker and Conyn-van Spaendonck, 1998). There was a linear increase of seroprevalence to virtually 100% in young adults (20-29 years of age), which was maintained in higher age groups. Back-calculation of these results indicated frequent exposure to *Campylobacter* is necessary to maintain this high level of sero-positivity. Recent data from the Work Package 32 of the Med-Vet-Net Network of Excellence (Falkenhorst, 2009) suggest that in seven MS (Finland, Denmark, the Netherlands, Italy, Romania, France and Poland), the sero-incidence varies between 600 and 900 infections per 1000 persons per year. There was little variation between MS, in contrast to results for *Salmonella* where the sero-incidence differed approximately 10-fold between MS with lower incidence rates in Nordic countries compared to continental Europe. There was no correlation between sero-incidence and laboratory-confirmed cases of campylobacteriosis. The relationship between circulating antibodies and immune protection is, as yet, unknown. Nevertheless, similar infection rates are suggested by risk assessment studies in which exposure to *Campylobacter* by all sources was modelled (Evers et al., 2008). Combining all information leads to a reconstruction of the surveillance pyramid in the Netherlands, as shown in Table 2.

Table 2: The surveillance pyramid of campylobacteriosis in the Netherlands (Havelaar et al., 2009).

Cases	Incidence (per 100,000 person years)	Background information
Fatal	0.15-0.30	Extrapolation from Danish registry-based study
Hospitalized	3.5-4.0	Laboratory surveillance
Reported	35-45	Laboratory surveillance
Consulting general practitioner	90-150	GP-based study (NIVEL)
Non-consulting	400-600	Population-based study (Sensor)
Asymptomatic, sero-conversion	10,000-20,000	Sero-surveillance
Asymptomatic, infected	40,000-60,000	Risk assessment model

At the EU-level, the underascertainment ratio is expected to range between 10 and 100 in different MS. This would imply that in the EU27 (population on 1st January 2007 was around 500 million), the approximately 200,000 reported cases would translate into not less than 2 million and possibly as high as 20 million cases of clinical campylobacteriosis per year. A similar range of 2-5 million cases per year is suggested by the serosurveillance studies.

2.3.2. Sequelae and mortality

The main outcome of *Campylobacter* infection is acute enteritis. However, there may be sequelae following (recovery from) the acute symptoms including Guillain-Barré syndrome (GBS), reactive arthritis, irritable bowel syndrome, and inflammatory bowel disease (Gradel et al., 2009; Havelaar et al., 2000; Helms et al., 2003; Mangen et al., 2005; Smith and Bayles, 2007). Excess mortality after acute *Campylobacter* gastro-enteritis has also been documented (Helms et al., 2003). An analysis of all published evidence in this area indicates that *C. jejuni* infection precedes GBS onset in 20-50% of cases (Jacobs et al., 2008) and that the incidence of GBS per 100,000 population is 0.6-1.9. The combined impact of these different outcomes of infection can be quantified using integrated health metrics such as Disability Adjusted Life Years (DALYs).

In the Netherlands, *Campylobacter* causes the largest burden of seven food-borne pathogens, followed by *Salmonella* and *Toxoplasma*[11]. For *Campylobacter*, the largest contribution to the burden is from complications, in particular GBS. Mortality also causes a relatively high burden, while the contribution of acute gastroenteritis is relatively low (Molbak and Havelaar, 2008). Recent work (Haagsma et al., submitted for publication) suggests that approximately 9% of all enteritis cases go on to develop (post-infectious) irritable bowel syndrome (PI-IBS). This would imply approximately 7,000 new cases per year in the Netherlands, and would explain 10% of the total prevalence of formally diagnosed IBS. Although there are few data on the severity and duration of PI-IBS, it was estimated that PI-IBS doubles the total burden of *Campylobacter*-associated illness in the Netherlands, from approximately 1,600 to 3,000 DALYs per year. The costs of campylobacteriosis and sequelae (excluding IBS) in the Netherlands (80,000 cases per year, 40 fatal cases) were estimated at 21 million € per year (Mangen et al., 2005). In Belgium (55,000 cases), the costs were estimated at 27 million € per year (Gellynck et al., 2008). Extrapolating these estimates would result in a disease burden of 0.1-1 million DALYs per year for the EU27 and total costs between 0.5 and 5 billion € per year. The disease burden is mainly due to sequelae, whereas enteritis also contributes to the cost of illness.

[11] See http://www.rivm.nl/vtv/object_class/kom_voedsel_micro.html

2.3.3. Antimicrobial resistance

A joint opinion on antimicrobial resistance focused on zoonotic infections has been issued recently by ECDC, EFSA, EMEA and SCENIHR (EFSA, 2009d). This document highlights how antimicrobial resistance has increased worldwide in bacterial pathogens leading to treatment failures in human and animal infectious diseases and how resistance against antimicrobials by pathogenic bacteria represents a major concern in anti-infective therapies. The opinion gives an overview of existing knowledge on antimicrobial resistance, focusing on specific combinations of zoonotic bacteria and antimicrobials which are regarded as of highest concern for human health. Resistance of *Campylobacter* to fluoroquinolones and macrolides is therefore discussed on the basis of the burden of human campylobacteriosis and of the high importance of these two antimicrobial classes. Among other aspects, the opinion summarises the available knowledge in relation to the exposure of humans to the resistance through food. With regard to fluoroquinolone resistance in *Campylobacter*, broiler meat has been shown by several studies to represent one of the main risk factors.

Many studies have shown that fluoroquinolone resistance in campylobacters from humans follows the usage of fluoroquinolones in food animals and the corresponding build-up of resistance among campylobacters from food animals (Endtz et al., 1991; Nachamkin et al., 2002; Thwaites and Frost, 1999). Approval and use of fluoroquinolones in poultry followed by increases in fluoroquinolone resistance in *Campylobacter* spp. from poultry and from human clinical cases has been shown in The Netherlands (Endtz et al., 1991; Piddock, 1995), Spain (Saenz et al., 2000; Velazquez et al., 1995), UK (Kramer et al., 2000; Threlfall et al., 1999) and the US (FDA, 2001; Smith et al., 1999).

As indicated above (see Sections 2.2.2. and 2.2.3.), it seems that antimicrobial susceptibility patterns and information on the genetic background for the resistance might provide useful data when establishing hypotheses on sources of human infection, although this has not been systematically explored.

3. Human illness source attribution methods

A variety of general approaches to attribute food-borne diseases to specific sources are available, including microbiological and epidemiological approaches, intervention studies, and expert elicitations (EFSA, 2008a; Pires et al., 2009), as summarised in Table 3.

Table 3: Methodologies for attribution of human illness to specific sources

Approaches	Methods
Microbiological approaches	Microbial subtyping
	Comparative exposure assessment
Epidemiological approaches	Analysis of sporadic cases
	Analysis of data from outbreak investigations
Intervention studies	
Expert elicitation	

Human illness source attribution can take place at different points along the food chain (points of attribution), including at the level of reservoir, distribution and exposure. Because pathogens that cause food-borne disease may enter the food distribution chain at different points, the burden of illness caused by one disease attributed to specific sources may vary, depending on the point along the food chain at which the approach focuses. For example, attribution of *Campylobacter* infections may partition more illness to chicken at the point of production (reservoir) than to broiler meat at the point of consumption, since other foods, e.g. raw vegetables, may be cross-contaminated with the pathogen during processing or preparation in the kitchen. Some of the source attribution approaches work

primarily at one point in the food chain (e.g. epidemiological approaches work primarily at the point of exposure), while other methods (e.g. expert elicitation approaches) can be more generally applied. The method for source attribution chosen, and consequently the point of attribution, will depend on the availability of data and the risk management question being addressed.

Attribution of human illness to specific sources requires categorization of the sources. Harmonization of the categorization schemes is required for comparisons and integration of results from various models and approaches. The main reasons for this requirement include (1) the categorization of sources often varies according to the source of the data (e.g. the collection and compilation of data from different institutions and different countries); (2) data may correspond to different points of the transmission chain, describing the reservoir, processing or exposure levels (a source at the reservoir level will often include several sources/routes of exposure in the processing or exposure level); and (3) integration of source attribution approaches and comparison of results from different methods are often used to overcome the limitations of the approaches and improve our knowledge on the most important sources of a specific pathogen. A categorization system should be hierarchical, while accommodating different levels of detail required for different purposes. Furthermore, the categorization schemes should agree with existing international animal-data and food-consumption databases. Exposure to pathogens commonly transmitted through foods can be categorized in five main groups: food-borne, contact with animals, environmental, person-to-person, and travel (i.e. exposure to any of these sources outside the country of residence, see also Section 2.2.3).

To identify sources and evaluate trends of food-borne disease, it is imperative to distinguish between sporadic cases and outbreak-associated cases. In some circumstances, cases classified as sporadic may belong to undetected outbreaks. The relative contribution of each food type to sporadic and outbreak-associated disease may differ, and extrapolation from the outbreak situation to sporadic disease should be made with care. Certain vehicles may be more likely to be implicated in outbreaks than others, especially if investigators preferentially collect data on the types of food perceived as high risk, or when laboratory methods vary in sensitivity according to food type. A systematic vehicle detection bias might underestimate the contribution and risks attributable to foods less commonly implicated in outbreak investigations, e.g. salad items, fruits, or background ingredients such as herbs and spices. In addition, it is important that large outbreaks are detected.

4. Attribution through microbiological approaches

4.1. Microbial subtyping

4.1.1. Methodology

The use of microbial subtyping is a major approach to attribution of infectious diseases including gastroenteritis (Batz et al., 2005; Havelaar et al., 2007). The principle of the microbial subtyping approach is to compare the subtypes of isolates causing human disease with the distribution of the same subtypes isolated from different sources (e.g., animals, food). The microbial subtyping approach involves characterization of isolates of a pathogen by phenotypic and/or genotypic subtyping methods (e.g. serotyping, phagetyping, antimicrobial susceptibility testing, pulsed-field gel electrophoresis (PFGE) or sequence-based subtyping, such as multi-locus sequence typing (MLST)). Ideally attribution would be most reliable when the particular subtypes from each source were unique to that source. Then human infections caused by subtypes found in several sources could be assigned to specific sources proportional to the occurrence of the indicator subtypes. The method also works with the less restrictive assumption that different sources harbour different distributions of subtypes. In combination with the availability of substantial data on the distribution of subtypes in different sources and incidence by subtype in humans it has been possible to estimate the source of human infections probabilistically with regard to food animal species and whether domestically produced or imported. The application of this approach assumes that the distribution of subtypes in the collection

of isolates in each source is representative of the true distribution of subtypes in each source. Because the microbial subtyping approach utilizes a collection of temporally and spatially related isolates from various sources, it is facilitated by an integrated food-borne disease surveillance programme that is focused on the collection of representative isolates from the major food animal reservoirs of food-borne diseases as well as isolates representative of the human cases.

Inclusion of information regarding exposure to the source, differential virulence and survival rates in the food chain may improve the quality of the results (Hald et al., 2004). It is also important to obtain information about the number of travel and outbreak-related cases so that they can either be removed from the analysis or dealt with in a different manner (EFSA, 2008a).

A feature of the microbial subtyping approach, which can be a limitation, is that it identifies the original (typically zoonotic) source and does not usually help in assessment of the route of infection, such as cross-contamination during processing.

4.1.2. *Campylobacter* attribution using subtyping

Studies have been mostly performed on *C. jejuni*. Compared to *Salmonella*, until recently, no similar success has been reported with *Campylobacter* subtyping despite extensive work using a wide range of subtyping approaches.

Although there has been a clear progression from phenotyping to non-sequence genotyping to sequence-based subtyping, all methods continue to be used. Phenotyping studies have identified that human isolates are more closely related to those from farm animals than wild animals (Devane et al., 2005; Garrett et al., 2007), with one identifying most similarity with isolates from broilers, albeit this effect was small (Rosef et al., 1985). Difficulties in the reproducibility and standardisation of phenotyping (Frost et al., 1998; Hanninen et al., 1999), the presence of a high proportion of untypable isolates (Wassenaar and Newell, 2000) and evidence that phenotype is not linked to other more robust measures of relatedness (Hopkins et al., 2004) have contributed to a general decline in the application of phenotypic methods. Among the non-sequence-based genotyping methods, PFGE and amplified fragment length polymorphism (AFLP) are the two that have been most widely applied to source attribution. Studies in Sweden and Denmark on PFGE subtypes indicated that human isolates are more closely linked to those from chickens (and in particular broilers) than from wild birds or mammals (Broman et al., 2002; Broman et al., 2004; Petersen et al., 2001). Although PFGE can be standardised, this requires substantial resources (Gerner-Smidt et al., 2006; Ribot et al., 2001) and is not generally done. The consequence is that it is not possible to combine the data from the above PFGE studies for joint analysis. Five AFLP studies have assessed host association and possible sources of human infection but there is little evidence for the capacity of this method to predict source (Duim et al., 1999; Hopkins et al., 2004; Schouls et al., 2003; Siemer et al., 2004; Wieland et al., 2006).

Recently, with the availability of *Campylobacter* genome sequences, microarray approaches have been developed. One such study identified livestock and non-livestock clades with most human cases fitting into the livestock clade (Champion et al., 2005). This study lacked an adequate sample for non-human isolates, used an array based mainly on a single genome sequence, and did not take into account the population structure already identified by sequence-based subtyping methods (Dingle et al., 2002; Dingle et al., 2001). These microarray observations have not been reproduced by other complimentary sequence-based studies (Karenlampi et al., 2007). Microarray studies in *Campylobacter*-related organisms (i.e. *Helicobacter* spp.) indicate a lack of congruence with unbiased sequence-based typing methods using the core genome (Gressmann et al., 2005).

With advances in DNA sequencing technology, sequence-based typing has become a popular approach. In particular, MLST has been developed as a generic typing method, which has considerable advantages in terms of reproducibility and portablity. For *Campylobacter*, MLST

involves sequencing seven gene fragments (Dingle et al., 2001; Maiden, 2006; Maiden et al., 1998). These gene fragments have been chosen to allow (1) the subtyping of all isolates and (2) the identification of the genetic relatedness between isolates. The genes chosen code for essential metabolic functions, i.e. they are "house-keeping" genes. This means that they are likely to be present in all isolates and that they are under stabilising selection, which limits the diversity available from each gene fragment. Closely-related isolates, sharing identical alleles at several of the loci, are thus identified as belonging to a particular clonal complex (CC) or even having an identical sequence subtype (ST) across all seven loci. Isolates identified as belonging to the same CC are very likely to share a more recent common ancestor than do isolates which are not members of the particular clonal complex (Maiden et al., 1998; Smith et al., 1993). Further information on the methodology of MLST and the analysis of the data generated is given in Appendix A.

With increased understanding of the population biology of the organism, some reasons for the difficulties encountered in *Campylobacter* subtyping attribution approaches have become clearer.

Firstly *C. jejuni* is highly diverse. This is demonstrable with every typing method used (Gerner-Smidt et al., 2006; Ribot et al., 2001) but the degree of diversity has become much clearer using MLST. The population structure of *C. jejuni* is termed "weakly clonal", which indicates that there are some clonal complexes or lineages in which the isolates are considered to be derived from a common ancestor. Large collections of isolates have now been subjected to MLST, which has demonstrated substantial diversity with 4218 unique sequence subtypes (STs) identified in the combined *C. jejuni* / *C. coli* pubMLST database[12] (at 25 November 2009). This means that there are many rare subtypes; large numbers of isolates from each possible source are therefore needed to give an accurate estimate of their differential distribution. This dataset provides a catalogue of diversity and is not representative of the distribution of these STs because it may be biased due to individuals submitting solely new types to PubMLST. The Scottish clinical dataset (Forbes et al., 2009) is a large representative dataset from human disease. This dataset comprises 5,247 isolates composed of 609 STs. The ten most common STs comprise 45.7% of the isolates, whilst singletons comprise 7.1% and STs occurring between two and ten times make up 12% of the dataset. This confirms this picture of substantial diversity, with many different rare types represented.

Secondly, genetic recombination of chromosomal DNA occurs frequently in *C. jejuni* (Dingle et al., 2001; French et al., 2005; Manning et al., 2003; McCarthy et al., 2007; Schouls et al., 2003; Suerbaum et al., 2001). As a result two isolates which are not closely related can be identical for a specific genetic region, while two closely-related isolates may be very different for the same region. This means that subtyping methods for *C. jejuni* need to measure many characteristics of each isolate to allow reliable assessment of how closely related isolates are to each other or evaluation of the probability that two subtypes are the same.

Thirdly, due to recombination between lineages within host species, methods of clustering isolates, based on identification of those from the same ancestral lineage, may not be efficient in identifying origin so that analytical approaches considering genes that have been taken up from other lineages may be needed (McCarthy et al., 2007).

Finally, when attributing the source, other features specific of *Campylobacter* epidemiology need to be considered, such as the fact that *Campylobacter* exposure involves both food-borne and environmental pathways. This is further complicated by dependence on both the age of individuals in the human population under study as well as the geographical location in which they live.

The above findings regarding the biology of this bacterial species mean that (1) analyses identifying a single overall subtype for isolates will not work reliably in determining source; (2) analytical methods

[12] See http://pubmlst.org

that are based on subtypes being identical or from the same lineage will not be efficient and (3) large reference populations will be needed from each possible source for this bacterial species.

Efficient attribution for *C. jejuni* therefore requires a subtyping method that is easily standardised so that data from studies can be combined to build shared sets of reference data. It also requires data that can be analysed using population genetic approaches that consider both ancestral relatedness and imported genes. DNA sequencing is inherently standardised and sequencing of multiple genes or gene fragments has become well established as a method to study the population biology of bacteria (Maiden, 2006; Maiden et al., 1998) including *C. jejuni* (Dingle et al., 2001; French et al., 2005; Manning et al., 2003; McCarthy et al., 2007; Schouls et al., 2003; Suerbaum et al., 2001). The widespread application of the set of seven gene fragments from a common MLST scheme (Dingle et al., 2001) has provided substantial data from separate studies that can be analysed jointly. Analytical approaches have been developed and allow attribution to source based on population genetic approaches to which multi locus DNA sequence data is particularly suited (McCarthy et al., 2007; Wilson et al., 2008). These have been applied using data from multiple studies (Sheppard et al., 2009). Both theory and practice currently support the capacity of an MLST approach to allow substantially more extensive and robust inference in subtyping based attribution than earlier subtyping approaches. Sequence subtyping is becoming less expensive so that much more extensive reference collections of isolates with this amount of sequence data are likely to become available in the short term. In the longer term (over the coming decade) the reduced cost of DNA sequencing is likely to lead to many studies generating far more data per isolate than the current standard seven gene fragments in MLST. Existing work shows that more extensive data will allow improved accuracy of subtyping (McCarthy et al., 2007). This will complicate the easy joint analysis of data from investigations which all study the same seven MLST gene fragments. Unlike earlier subtyping methods, which were not generally backwards-compatible, genetic sequencing and population genetic approaches allow joint analysis of data where some isolates have additional, sequence information. Similarly, developments of the database approaches currently used to share MLST data (Jolley et al., 2004) can support large publicly-accessible isolate collections with much more extensive genetic sequence data per isolate. The current seven gene MLST and the move to use population genetic approaches, informed by the biology of *C. jejuni* to analyse this data (McCarthy et al., 2007; Sheppard et al., 2008; Wilson et al., 2008), is therefore a step in what should be a more coherent evolution of the subtyping of *C. jejuni*.

A detailed description of available phenotyping and genotyping methods and of their application for source attribution purposes is provided in Appendix A.

4.1.3. Implementation of *Campylobacter* subtyping methods in the EU

In order to gather data in relation to the subtyping methods implemented in the different countries throughout the EU and some nearby third countries, EFSA developed a questionnaire survey, results from which have recently been published (EFSA, 2009e). The questionnaire was distributed to all EU MSs and to five non-MSs and concerned molecular subtyping methods used for different food-borne pathogens (i.e. *Salmonella*, *Campylobacter*, verotoxigenic *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus*) from animals, food and feed.

Regarding the molecular subtyping of *Campylobacter* in the EU, 19 MS indicated that they perform molecular subtyping for animals, food and feed isolates, while 6 MS declared that they do not perform molecular subtyping or that they purchase the service from other countries. In particular, 18 MS perform the subtyping of animal isolates (4 routinely and 14 occasionally). Similarly18 MS perform the subtyping of food isolates (4 routinely and 14 occasionally).

The origin of the samples varies depending on the countries. Fifteen MS indicated the origin is official controls, national controls, monitoring programmes or surveys carried out by competent

authorities. In addition, 13 MS also type isolates in the framework of outbreak investigations and 17 MS type isolates in the framework of research programmes.

Subtyping methods used by the different countries also differ. PFGE was the main method used for molecular subtyping of *Campylobacter* isolates, as declared by 12 of the 19 MS, while MLST was used by 8 MS. Twelve MS also used other molecular methods to subtype *Campylobacter* isolates.

ECDC is currently carrying out a similar survey with regard to these methods used for subtyping isolates of *Campylobacter* from cases of human infection in the different EU MS and the results should be available shortly.

4.1.4. Modelling attribution approaches based on microbial subtyping

There are five main mathematical modelling approaches that have been developed for attributing disease on a population level using microbial subtyping. The methods are described in detail in Appendix B, but below is a brief outline of the techniques (starting broadly from the simplest and progressing to the most complex), together with results from published studies as well as an assessment of the relative strengths and weaknesses of each. Table 4 summarises the results from published studies using modelling attribution approaches based on MLST subtyping.

4.1.4.1. The Dutch Model

The Dutch Model (French and the Molecular Epidemiology and Veterinary Public Health Group, 2008; Van Pelt et al., 2006) is a straight forward way to estimate the attribution of a particular genotype (e.g. ST) to a reservoir, when the frequency distribution of each subtype is known for each reservoir. When applied at the ST level this model does not guarantee that all STs will be attributed to sources. This is because human subtypes which are not found in the animal reservoir cannot be attributed. However, if genetic information exists at multiple loci (e.g. 7 loci for this study), then the Dutch Model can make use of the frequency of each individual allele at each individual locus, and estimate attribution even for STs which are not present in the source population.

The Dutch Model does not take into account the uncertainty in the frequency distribution of genotypes. Neither does this model consider any information about the exposure of humans to sources or the viability/virulence of pathogens once they are ingested by humans. The Dutch model has been used at the ST level to analyse the New Zealand MLST data (French and the Molecular Epidemiology and Veterinary Public Health Group, 2008). This model attributed 52% of isolates that it was capable of classifying as originating from poultry.

4.1.4.2. Population STRUCTURE

STRUCTURE (Pritchard et al., 2000) is a Bayesian clustering model designed to infer population structure and to attribute individuals to population groups. The program readily uses MLST genotyping data. Each isolate is attributed on the basis of a training dataset consisting of isolates from known populations. STRUCTURE can be used at the ST or allele levels, it incorporates uncertainty and takes account of sample size. Hence, in principle, it gives a more realistic estimation of the attribution to a specific reservoir than the Dutch Model. Also, it can assign human cases that have STs which are not found in the animal reservoirs. However, it is highly time consuming and does not explicitly consider any differences in exposure to risk factors/reservoirs or to differing infectivities of subtypes.

STRUCTURE has been used to attribute clinical *Campylobacter* strains from Scotland with reservoir data from the PubMLST database (Sheppard et al., 2009). It reported that 58% and 40% of *C. jejuni* and *C. coli* respectively could be attributed to chicken. A further study into *Campylobacter* in young

children in North-east Scotland (Strachan et al., 2009) attributed 43% and 19% of cases to chicken in urban and rural areas, suggesting that the relative importance of the sources of *Campylobacter* infection in young rural children may be different to their urban counterparts. The above results were obtained by analysis at allele level, which are considered to be more robust than analysis at ST level (McCarthy et al., 2007).

4.1.4.3. Hald Model

A more advanced application of the microbial subtyping approach was developed in Denmark for the attribution of human salmonellosis (Hald et al., 2004). Using data from the integrated Danish *Salmonella* surveillance programme, a mathematical model was developed to quantify the contribution of each of the major food animal sources to human *Salmonella* infections. The "Danish *Salmonella* source attribution" model uses a Bayesian framework with Markov Chain Monte Carlo simulation to attribute sporadic laboratory-confirmed human *Salmonella* infections caused by different *Salmonella* subtypes as a function of the prevalence of these subtypes in animal and food sources and the amount of each food source consumed. The model takes into account the uncertainty for all these factors and also includes travel as a possible risk factor.

This microbial subtyping approach has proved to be a valuable tool in focusing food safety interventions, primarily *Salmonella*, to the appropriate animal reservoir in Denmark and provides an example of potential synergy between quantitative risk assessment and public health surveillance (Hald et al., 2004). The model has also been successfully applied to *Salmonella* in a number of European countries (Pires et al., 2008). Although this is a more advanced model than the others described above, it is dependent on the amount of data available. Variables relating to bacteria-dependant factors and host-dependant factors can be estimated more reliably if data from several years are taken into account (Pires and Hald, 2010).

4.1.4.4. Modified Hald Model

This model is a variation of the Hald Model that has been developed for use with both *Salmonella* and *Campylobacter* (Mullner et al., 2009a). The modifications include: removal of the food factor variables, incorporation of the uncertainty in prevalence of the source reservoirs and incorporation of environmental sources.

This model has been applied to MLST data from Manawatu in New Zealand (French and the Molecular Epidemiology and Veterinary Public Health Group, 2008) where it was found that 67% of human cases could be attributed to poultry. This model has advantages over the original Hald model in that it incorporates uncertainty in reservoir prevalence and also improves identifiability of variables. However, it does not explicitly incorporate exposure and operates at the ST level only so that human subtypes absent from the source reservoirs cannot be attributed.

4.1.4.5. Asymmetric island (AI) model

The Asymmetric Island Model (Wilson et al., 2008) incorporates a Bayesian approach and uses the allelic profile of the sequence subtypes to reconstruct the genealogical history of the isolates. The host populations are considered to exist on separate "islands" (e.g. the island sheep). Mutations and recombination occur on each island. Migrations from between each reservoir (island) and into the human population are used to estimate the degree of attribution to each source.

This model has been applied to MLST data from England, Scotland and New Zealand where 56.5%, 78% and 75% of human cases were attributed to poultry respectively.

The AI model incorporates recombination and mutation, uses MLST data at the allele level and achieves relatively high values for self attribution. However, the model appears to be complicated and the current explanations of its operation are difficult to comprehend. The AI model assigns each human case to the potential source populations on the basis of DNA sequence similarity. By comparing human isolates to a panel of reference sequences of known source (e.g. cattle, sheep, chickens, pigs, wild birds and the environment), each human case can be assigned a probability of originating in each source population. The source attribution probabilities are calculated using a statistical model of the way the DNA sequences evolve in the populations of bacteria. In the statistical model, there are parameters representing the processes of mutation, DNA exchange between bacteria (recombination or horizontal gene transfer) and zoonotic transmission between populations. These processes lead to differences in gene frequencies between the source populations, facilitating source attribution. The model can be trained, by estimating the parameters exclusively from the sequences of known source, before using it to calculate source attribution probabilities for human sequences.

4.1.4.6. Self attribution in source attribution modelling

A key performance factor of these models can be termed "self attribution". This is the average percentage accuracy that any given isolate from a reservoir can be correctly attributed back to its reservoir. This can be performed in a number of ways. However, one simple approach is to use a jacknife method to predict the source of an isolate that was unknown to the model and known to the user. This is then repeated for all the source isolates a number of times (e.g. up to 10,000) so that an average, and confidence intervals, can be calculated. Self attribution ranges between 62-97% for between 5-7 hosts for the asymmetric island model (Sheppard et al., 2009; Wilson et al., 2008) and 38-70% for STRUCTURE (Sheppard et al., 2009). Note that by chance you would expect a correct self attribution of 20% and 14% for 5 and 7 sources respectively. The poorest self attribution in these methods is environment, which is likely to contain isolates from a number of hosts. These data demonstrate that there are differences in the frequencies of MLST types between hosts and that this information can be used for source attribution.

Table 4: Summary of published studies using MLST subtyping for source attribution

Attribution Model	Source Animal Dataset	Clinical Dataset	Species	% Attribution to chicken	% Attribution to Other Sources	Comments	Reference
Asymmetric Island	1145 isolates from 10 previous studies	1255 from NW England; Jan 2000 till Dec 2002	*C. jejuni*	56.5	35.0 (cattle) 4.3 (sheep) 2.3 (wild animals) 1.1 (environment)		(Wilson et al., 2008)
Population structure	5247 from Scotland July05 to Sept06 (9.6% *C. coli*)	999 From Scotland and 3419 from PubMLST	*C. jejuni*	58	38 (ruminants) 4 (wild bird & environment)		(Sheppard et al., 2009)
Asymmetric Island	As above	As above	*C. jejuni*	78	38 (ruminants) 4 (wild bird & environment)		(Sheppard et al., 2009)
Population structure	As above	As above	*C. coli*	40	40 (sheep) 14 (cattle) 6 (pigs) 1 (turkey)		(Sheppard et al., 2009)
Asymmetric Island	As above	As above	*C. coli*	56	40 (sheep) 2 (cattle) <1 (pigs) <1 (turkey)		(Sheppard et al., 2009)
Population structure	680 contemporaneous isolates from Scotland	225 from rural children in Grampian 2000-06	*C. jejuni* and *C. coli*	19	42 (cattle) 24 (wild birds) 12 (sheep) 3 (pigs)	Rural children <5 years	(Strachan et al., 2009)
Population structure	As above	85 from urban children in Grampian 2000-06	*C. jejuni* and *C. coli*	43	35 (cattle) 6 (wild birds) 15 (sheep) 1 (pigs)	Urban children <5 years	(Strachan et al., 2009)
Modified Hald	793 isolates	481 from Manawatu, New Zealand	*C. jejuni*	80	10 (cattle) 9 (sheep) 4 (environment)		(Mullner et al., 2009a)

Dutch Model	521 isolates	As above	*C. jejuni*	52	17 (cattle) 10 (sheep) 5 (wild bird) 11 (water)	(French and the Molecular Epidemiology and Veterinary Public Health Group, 2008)
Modified Hald Model	521 isolates	As above	*C. jejuni*	67	23 (cattle) 8 (sheep) 1 (wild bird) <1 (water)	(French and the Molecular Epidemiology and Veterinary Public Health Group, 2008)
Island Model	521 isolates	As above	*C. jejuni*	75	17 (cattle) 4 (sheep) 2 (wild bird) <1 (water)	(French and the Molecular Epidemiology and Veterinary Public Health Group, 2008)

4.2. Comparative exposure assessment

As discussed in the EFSA scientific opinion on "Overview of methods for source attribution for human illness from food borne microbiological hazards" (EFSA, 2008a), a recent development in attribution methods is the application of risk assessment methodologies to quantify exposure to pathogens from a multitude of sources. Current methods estimate exposure per person per day, averaged over a specified population (e.g. all inhabitants of one country). Exposure is estimated separately for all relevant specific sources that can be part of categories such as food, animal contact and environment. Results of all exposures can be accumulated to calculate the total exposure (and to produce a risk estimate) or can be ranked to identify the most significant sources of exposure.

An example of the approach is a Dutch exposure assessment for *Campylobacter* spp. (Evers et al., 2008). These authors estimated the mean dose of *Campylobacter* ingested per person per day averaged over the entire Dutch population by different routes including consumption of food (animal or vegetable origin; raw or prepared), direct contact with animals (pets, farm animals and petting zoo animals) and water (swimming in or drinking water). Thirty-one routes related to these categories were investigated. Approximately two thirds of the average exposure was related to direct contact with animals, whereas only one third was related to food. Surface water contributed only 1% to the total exposure. Within the food routes, raw or partly cooked foods (chicken liver, milk, herring, and vegetables) were the major sources of exposure, with chicken meat being the most important source of exposure from cooked meats.

Lake et al. (2006) has made a similar analysis for New Zealand; a country that has a very high reported rate of campylobacteriosis. Results were not based on a formal mathematical model but, by taking information from human disease surveillance and exposure information into account, the author concluded that poultry meat is an important source of human campylobacteriosis in New Zealand. Of other risk factors, overseas travel and animal contact (for the rural population) appeared to be most important. Other risk factors, which were considered of lesser importance, were red meats, offals, raw milk, drinking water, environmental waters, and pets. McBride et al. (2005) used a model similar to that of Evers et al. (2008) to compare infection risks from four major pathways (food, recreational swimming, drinking water and occupational contact with livestock). In this study, the mean estimated daily exposures were combined with a dose-response model to estimate infection risks. It was concluded that cross-contamination from poultry and, to a lesser extent, from red meat were the exposures that most frequently caused infection. This was followed by occupational exposure, drinking untreated water and recreational swimming. As in the Dutch study, there were major uncertainties in the data underlying the model and the results are considered preliminary. Nevertheless, there are important differences between the results from the two studies, in particular in the importance of direct animal contact. Further work, both to improve the data underlying the model, and to develop the modelling approaches, are necessary before conclusions can be drawn.

5. Attribution through epidemiological approaches

Epidemiological studies usually involve interviews of patients to elicit the patient's recall of foods consumed or other exposures before illness began. A variety of epidemiological methods can be used for human illness source attribution, including studies of sporadic infections and analysis of data from outbreak investigations. Studies can be built upon an existing public health surveillance infrastructure or involve additional interviews of patients and asymptomatic (control) individuals.

5.1. Analysis of sporadic cases

Several types of studies have been performed to identify possible sources of apparently sporadic human infections, including case-control, cohort and case-series studies. This section focuses only on

case-control studies of sporadic infections, the most commonly used analytical epidemiological studies for identifying possible exposures.

To allow for sufficient enrolment of patients, case-control studies of sporadic infections are often conducted over an extended period of time, and commonly use public health surveillance to ascertain culture-confirmed cases. Selected case-patients and a corresponding group of asymptomatic, and therefore assumed to be uninfected, individuals (controls) are interviewed, and the relative role of exposures is estimated by comparing the frequency of exposures among cases and controls. When infections are associated with an exposure, the proportion of cases attributed to the exposure can be calculated and is defined epidemiologically as the population attributable fraction (PAF) (Clayton and Hills, 1993). The population attributable fractions can be used to partition the human disease burden to specific sources (Stafford et al., 2008).

Case-control studies are a valuable tool to identify potential risk factors for human infections, including sources, predisposing, behavioural and seasonal factors (Engberg, 2006). The studies that published estimates for the most important risk factors suggest that between 24 and 29% of infections can be attributed to the consumption of chicken meat (Stafford et al., 2007; Wingstrand et al., 2006). When estimates referring to chicken prepared or eaten at a restaurant are included, PAF range from 10% to 38% (Kassenborg et al., 2004; Unicomb et al., 2008). Consumption of unpasteurized dairy products has been estimated to be responsible for 1.5% to 4% of infections (Friedman et al., 2004; Neimann et al., 2003), and between 5.4% and 24% of campylobacteriosis were attributed to international travel (Friedman et al., 2004; Fullerton et al., 2007; Neimann et al., 2003). PARs for cases attributed to contact with farm animals ranged from 2% to 16% (Friedman et al., 2004; Fullerton et al., 2007; Neimann et al., 2003), and frequent contact with dogs from 2% to 30% (Carrique-Mas et al., 2005; Neimann et al., 2003).

In addition to individual case-control studies, a systematic review of published case-control studies of sporadic infections of a given pathogen can provide an overview of the relevant exposures and risk factors for that infection, and a summary of the estimated PAFs for each exposure.

With the objective of identifying the most important risk factors for campylobacteriosis, a systematic review (SR) of case-control studies of sporadic infections was undertaken under the scope of the Med-Vet-Net's Work Package 28, which focused on the development of methods for the attribution of food-borne illness (Domingues et al., 2009). Collected data was utilized in a meta-analysis to estimate the relative importance of extracted risk factors for *Campylobacter*.

The formal process for literature review established for SRs was followed (Sargeant et al., 2006). All collected risk factors were stratified according to pre-defined source-categorizations schemes, and a meta-analysis was conducted within each risk factor stratum to estimate a pooled odds ratio. Following the initial meta-analysis, a set of sub-analyses to evaluate the impact of the inclusion of studies from different regions in the world, time periods and age groups was performed. More detailed information on the methodology used for this study can be found in Appendix C.

Collected studies were conducted between 1983 and 2004 in 13 different countries belonging to 3 different continents. Most studies were designed to investigate exposures to *Campylobacter* in general; 8 studies focused on *C. jejuni*, and no study was restricted to *C. coli* infections. Consequently, no differentiation between species was undertaken in the analyses. Two case-control studies were performed specifically in rural areas and two in urban areas. This limited number of studies did not allow for sub-group analyses for rural and urban populations. Seven studies investigated exposures in children, and two focused only on adult age groups. Overall, the number of cases and controls interviewed varied substantially between studies, and both small scale and community studies were included. A list of all collected case-control studies of sporadic campylobacteriosis is given in Appendix C.

The overall meta-analysis for sporadic campylobacteriosis estimated that travel is the most important risk factor for disease. Travel-related cases in a European context, and travel as a risk factor, have been discussed in Section 2 of this opinion. Other important risk factors identified were daily contact with a pet and consumption of undercooked chicken (see Figure 3). Results suggested that consumption of chicken, other poultry, sausages, and chicken in a restaurant are the most important food-related risk factors. In addition, poor food-preparation hygiene was revealed to be a significant risk factor for infection. Direct contact with pets and farm animals, environmental, and person-to-person transmission, as well as predisposition factors, were estimated to be risk factors for disease. A sub-group analysis for children estimated that international travel, drinking unpasteurized milk, drinking untreated water, and direct contact with a pet are important risk factors in this age-group. A northern European analysis provided results similar to the ones obtained in the overall analysis, reflecting the substantial contribution of data from studies conducted in this European region to the overall analysis. Other European regions did not provide sufficient information for sub-analyses. As mentioned above, the limited number of case-control studies differentiating between species precluded separate meta-analyses for *C. jejuni* and *C. coli*, but a recent case-control study estimated that the consumption of chicken was a predominant risk factor for *C. jejuni*, whereas consumption of tripe and game and swimming were unique risk factors for infection with *C. coli* (Doorduyn et al., 2009). It is likely that, with increasing insight in the diversity of *Campylobacter*, risk estimates may need to be developed for separate species or even subgroups within species, as is now common practice for *Salmonella* serovars.

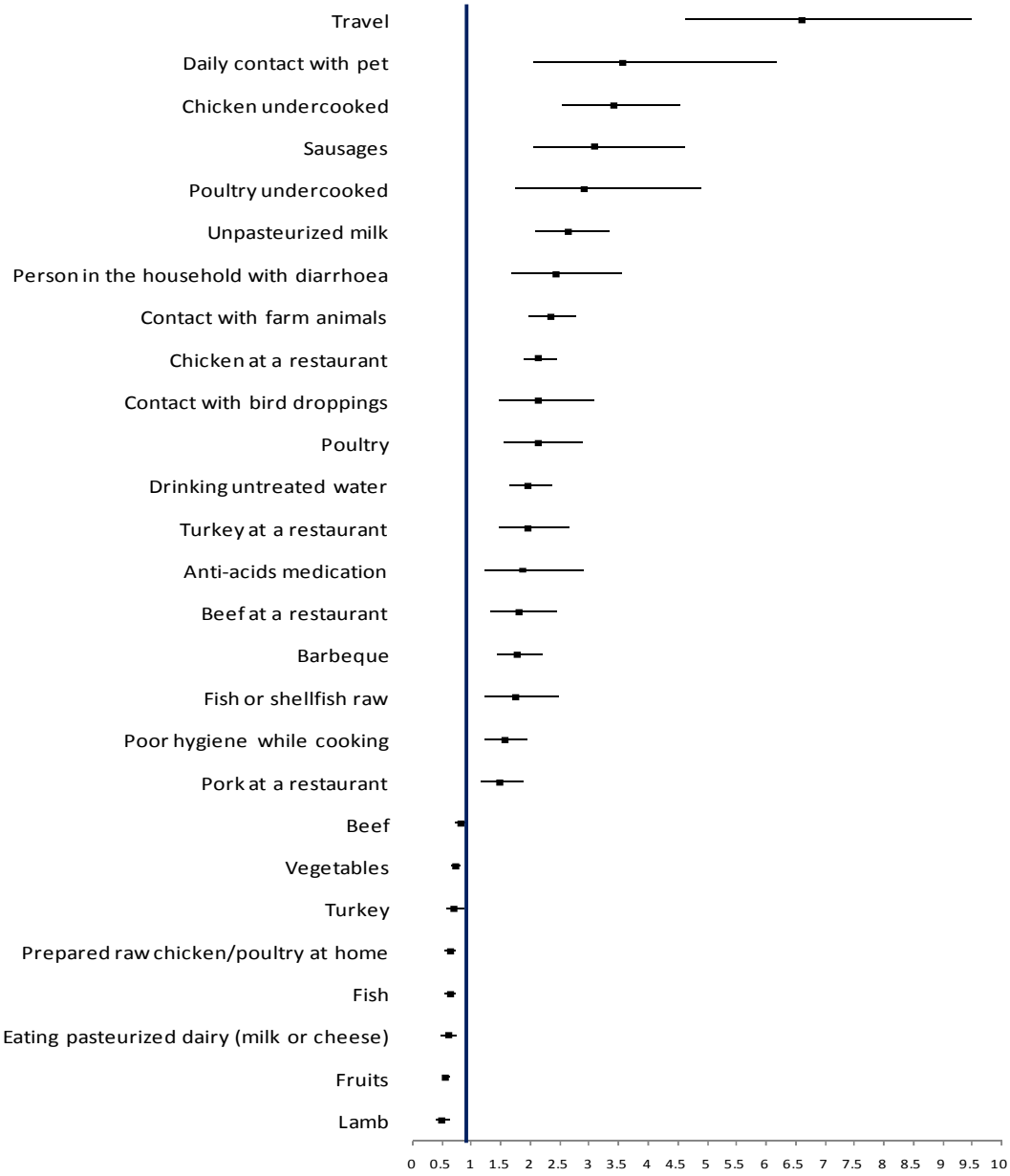

Figure 3: Relative importance of risk factors for sporadic campylobacteriosis (odds ratio and 95% CI), overall analysis (Domingues et al., 2009).

5.2. Analysis of data from outbreak investigations

The investigation of food-borne disease outbreaks has proven to be a critical means of identifying new agents and new vehicles, as well as maintaining awareness of contemporary problems, such as *Campylobacter* infections. The prompt and thorough investigation of food-borne outbreaks is particularly useful for timely identification of etiologic agents, sources and vehicles, and enables the prevention of further cases by identifying and eliminating the source of infection (Havelaar et al., 2007). Outbreak investigations generally involve both descriptive and analytical epidemiological studies, and are regularly combined with microbiological subtyping methods to support the results of

the epidemiological studies. Data from outbreak investigations are observed at the public health endpoint and may be a direct measure of attribution at the point of exposure. The trace back of a food-borne outbreak may allow the identification of the specific point of contamination in the food chain. The use of outbreak data for the attribution of human food-borne disease has been previously described (Adak et al., 2005; Greig and Ravel, 2009; Painter, 2006).

Many outbreak investigations have successfully identified the source of human infection. By conducting an analysis of data from outbreak investigations, the most common food vehicles involved in outbreaks can be identified. A simple analysis or summary of outbreak investigations is useful for attributing illnesses to foods, but often the implicated item consumed in an individual outbreak is a "complex" food, containing several components, many of which could be the specific source of the infection.

As discussed in Section 2.1, *Campylobacter* outbreaks appear to be relatively rare and outbreak-related cases seem to contribute only to a small proportion of the total burden of infection (Frost et al., 2002; Pebody et al., 1997) compared to sporadic cases. Therefore, the extent to which the overall disease burden can be attributed based on the source of infection in outbreaks requires firstly that sporadic and outbreak cases share the same sources, secondly that sufficient data is available from investigated outbreaks to allow reliable estimation, and thirdly that outbreaks from different sources are similarly likely to be identified. There is some evidence against the first assumption, since raw drinking milk and contaminated drinking water have been identified as important causes of outbreaks of campylobacteriosis, whereas sporadic cases are more frequently associated with poultry products (EFSA, 2005b). The second assumption has been highlighted as a particular weakness in relation to *Campylobacter* (Adak et al., 2005), where outbreaks are relatively rare compared to the overall burden of disease. Whether the third assumption holds is speculative, but a substantial publication bias appears to operate with published outbreaks not generally representative of incident outbreaks (O'Brien et al., 2006). Despite these caveats, data from outbreak investigations may still allow the identification of recurrent sources of human infection.

Since 2005, reporting of food-borne outbreaks has been mandatory for the EU MS. Food-borne outbreak investigation and reporting systems are not harmonised within the EU, thus differences in numbers and types of reported outbreaks and causative agents do not necessarily reflect different levels of food safety between MS. Outbreaks are reported as either general outbreaks, affecting members of more than one private household, or as family outbreaks, affecting only members of a single household.

In a recent study, data from investigations of *Campylobacter* outbreaks in Europe in 2005 and 2006 (EFSA, 2006, 2007) was modelled to attribute human campylobacteriosis to specific sources (Pires, 2009).

In 2005, *Campylobacter* was identified as the cause in 494 of the reported outbreaks (9% of all reported outbreaks). In most outbreaks further speciation of the *Campylobacter* isolate was not performed (15%) or not reported (68%). Information on the location of exposure was available for 36% of the 494 reported *Campylobacter* outbreaks. Of these, the most commonly reported locations were private homes (52%) and restaurants (10%). In 6% of the outbreaks the location of exposure was not identified and in 58% the location was not reported (EFSA, 2006).

In 2006, *Campylobacter* was identified as the cause in 400 of the reported outbreaks (6.9% of all reported outbreaks). In total, 1,304 persons were affected by campylobacteriosis and 65 were admitted to a hospital. The majority of the *Campylobacter* outbreaks were reported by Germany and Austria, reporting 208 and 136 outbreaks respectively. The majority of the reported outbreaks were household outbreaks (71.0%) affecting approximately 50% of all persons with campylobacteriosis. Eleven countries reported 119 outbreaks due to *C. jejuni* and Austria reported two *C. coli* household outbreaks (EFSA, 2007).

When reporting data relating to 2007, a new reporting system was used by MS, according to that agreed by the EFSA Task Force on Zoonoses Data Collection (EFSA, 2009b). This new reporting system differentiates between possible outbreaks and verified outbreaks, the latter referring to outbreaks where evidence (laboratory detection of the causative agent and/or analytical epidemiological evidence) is sufficiently strong to support the link between human cases and a food vehicle. Detailed information is only collected from verified food-borne outbreaks. Nonetheless, countries interpreted the new reporting guidelines differently, and consequently collected data is not comparable between MS. Additionally, the new reporting system complicates the comparison between data from 2007 and data from the two previous years. As a result, in this study (Pires, 2009), only data from outbreak investigations from 2005 and 2006 were analysed for source attribution. Table 5 presents *Campylobacter* outbreak-related data from 2005 to 2007.

Table 5: Reported outbreaks caused by *Campylobacter* in the EU, 2005-2007

Year	Reporting countries	Outbreaks		Human cases	
		N	% of total	N	Admitted to hospital
2005	14 MS + 1 non-MS	494	9.0	2,478	150
2006	15 MS + 2 non-MS	400	6.9	1,304	65
2007 (possible outbreaks)	22 MS + 2 non-MS	461	8.2	n.a.	n.a.
2007 (verified outbreaks)	22 MS + 2 non-MS	29	--	244	19

n.a. = not available

All *Campylobacter* outbreaks reported by MS and non-MS that agreed with a common definition of outbreak (see Glossary) were used in the analysis, including confirmed and suspected outbreaks, as well as outbreaks where evidence for an implicated source was not provided (source unknown). Implicated foods were reported based on epidemiological or laboratory evidence. Information on the species of *Campylobacter* causing the outbreak was unavailable for around 70% of the reported outbreaks. The vast majority of the outbreaks with identified species were caused by *C. jejuni*. Hence, the analysis was performed for *Campylobacter* spp. i.e. not distinguishing between different species of the pathogen. Outbreaks were classified as general or household outbreaks, according to the setting of the outbreak. The main category "general outbreaks" included all outbreaks that took place outside a private household. Reported outbreaks associated with travelling abroad were analyzed separately and were not attributed to any of the specific sources. Food items were categorized in a hierarchical scheme. Foods that contained only one category were considered "simple foods", while foods that contained multiple categories were considered "complex foods". In the latter, outbreaks and outbreak-related illnesses cases were attributed to the specified food categories on the basis of the proportion of outbreaks implicated to each category in simple food outbreaks. More detailed information on the methodology used for attribution of illnesses due to complex food can be found in Appendix C. To account for potential regional differences within Europe, separate analyses for the four United Nations regions were performed.

The proportion of reported human illnesses attributable to specific sources was estimated on the basis of both the number of reported outbreaks of campylobacteriosis and the number of illnesses registered in those outbreaks. Results from the analysis based on the number of ill people were substantially different from those based on the number of outbreaks (Table 6). For both analyses, consumption of meat, especially chicken and other poultry, was the most important source of food-borne campylobacteriosis. However, based only on the number of illnesses, drinking water is the most important overall source of campylobacteriosis. Consequently, all further analysis was undertaken based on the number of outbreaks. Foods are categorised according to the scheme presented in Appendix C.

Overall 10.5% of outbreaks of human campylobacteriosis were attributed to the consumption of chicken (95% CI: 4.7-20.6%), 0.6% to turkey (95% CI: 0.1 - 1.4%) and 1.4% to poultry (unspecified). A total of 12.4% of outbreaks were therefore attributed to the general category "poultry". In total, the proportion of outbreaks attributed to "meat and poultry-meat" was estimated to be around 25.9%. Similarly 4.5% of the *Campylobacter* outbreaks were attributed to dairy products (95% CI: 2.2 – 8.0%), 2.0% to seafood (95% CI: 0.9 - 4.1), and around 1.1% to international travel (95% CI: 0.2 - 3.2). All the remaining investigated sources were estimated to be of minor importance for campylobacteriosis. Overall 63.8% of the outbreaks were attributed to an unknown source (95% CI: 42.4 - 80.2). Among outbreaks that could be attributed to a known source, around 29% was attributed to chicken, around 12% to dairy products and, when combining all outbreaks attributed to meat sources, around 68% could be attributed to the category "meat and poultry".

Table 6: Attribution estimates showing the proportion of outbreak-associated campylobacteriosis cases and outbreaks attributed to specific sources in Europe, in 2005 and 2006 (%). Foods are categorized according to the scheme presented in Appendix C.

	Median (proportion of number of ill)	**95% CI**	**Median (proportion of number of outbreaks)**	**95% CI**
Eggs	0.64	[0.19,1.58]	1. 30	[0.36,3.30]
Dairy	2.53	[1.35,4.70]	4.49	[2.24,8.01]
Meat (unspecified)	10.25	[5.34,19.76]	12.59	[6.69,21.14]
Poultry (unspecified)	2.04	[0.26,6.24]	1.36	[0.34,3.55]
Chicken	10.23	[4.67,20.39]	10.45	[4.66,20.55]
Turkey	1.20	[0.07,4.34]	0.57	[0.12,1.44]
Beef	0.00	[0.00,0.00]	0.00	[0.00,0.00]
Pork	2.68	[0.13,9.64]	0.91	[0.27,2.19]
Lamb	0.00	[0.00,0.00]	0.00	[0.00,0.00]
Game	0.00	[0.00,0.00]	0.00	[0.00,0.00]
Fruits nuts	0.098	[0.00,0.41]	0.11	[0.00,0.48]
Vegetables	0.11	[0.00,1.24]	0.18	[0.00,0.80]
Grains beans	0.11	[0.00,0.36]	0.23	[0.00,0.78]
Oils sugar	0.26	[0.00,1.49]	0.30	[0.00,1.19]
Seafood	0.95	[0.43,1.95]	1.99	[0.85,4.09]
Drinking water	17.58	[0.00,45.66]	0.34	[0.00,0.98]
Travel	44.0	[21.51,69.37]	1.09	[0.22,3.18]
Unknown	36.19	[0.12,14.32]	63.80	[42.41,80.20]

A sub-analysis by location of the outbreak suggests that dairy products are a more important source in general outbreaks than in outbreaks in a home environment. Surprisingly, a high proportion of cases was attributed to eggs in general outbreaks. The regional analyses revealed substantial variability in the importance of sources to human campylobacteriosis within Europe. In Northern Europe, drinking water was estimated to be an important source of disease, but no cases were attributed to this source in any other country. In Eastern Europe, travel was the single most important source of campylobacteriosis, while meat and chicken were estimated to be important sources in Southern Europe. The analysis of the hospitalized cases suggested that the most important sources of severe infections were chicken and international travel (see Section 2.2.3 for a discussion of international travel in a European context).

From the 376,454 *Campylobacter* infections reported in the study period, 39,225 were attributed to chicken and 46,530 could be attributed to poultry-meats (including chicken, turkey and other poultry). In total, 97,277 (24.5%) infections were attributed to meat and poultry products. This estimate was obtained by aggregating all cases attributed to all meat categories within this main group of foods (see categorization scheme in Appendix C).

6. Attribution through intervention studies

The outcomes of measures taken in order to reduce either the prevalence of *Campylobacter* at different levels along the food chain, or the prevalence of human campylobacteriosis cases, can also be used for attribution purposes. This is because the effects of such interventions can provide information on the role of the source at that level at which the measures have been applied. Measures applied for other purposes, such as to control or eradicate animal diseases or to protect consumers from specific food-borne hazards, can also provide useful information.

Published studies were identified from four countries, which have adopted major national strategies to reduce campylobacteriosis by prevention and control of *Campylobacter* in poultry.

In Iceland, in the year 2000, strict control measures were implemented along the whole food chain (farm to retail) and an intensive consumer education campaign was implemented. This strategic approach reduced campylobacteriosis cases in Iceland by approximately 70%, from 116 to 33 per 100,000 population (Stern et al., 2003). Subsequently, the notification rate in Iceland has decreased to below 10 cases per 100,000 population. Current control measures in this country include checking faecal samples from all flocks for *Campylobacter* 2 to 4 days before slaughter. If results are negative the flock is processed and distributed fresh, if positive the product is commercially frozen or further processed before distribution (Callicott et al., 2008).

In Norway, a similar Action Plan against *Campylobacter* in broilers was initiated in 2001 and has been modified several times since. The poultry industry uses data from this Action Plan as an incentive for farmers to improve biosecurity. The proportion of positive flocks at slaughter is generally low in Norway and varied between 3.3 and 5.7% in the years 2002-2007 (Hofshagen and Brunheim, 2006; Hofshagen and Kruse, 2005; Hofshagen and Opheim, 2007, 2008). In 2008, all flocks were sampled at a maximum of four days before slaughter and checked for *Campylobacter* (approximately 75% of all positive flocks will be detected by sampling at this point). All positive flocks detected before slaughter were either frozen for at least 3 weeks or heat-treated before sale. Due to this policy, a substantial decrease in the number of untreated *Campylobacter*-positive flocks entering the retail market was seen in the years 2002–2004 and persisted through 2005-2007. Interestingly this decrease in exposure to *Campylobacter*-positive birds was not reflected in a decrease in the number of domestic human cases, where a slight increase was seen from 2002 to 2003/2004, followed by a sharp increase in 2005. Thereafter a decrease was observed in 2006 and this level was maintained during 2007. However, in Norway, other sources of domestically acquired campylobacteriosis, particularly drinking untreated water, are considered to be more important than broilers (Kapperud et al., 2003).

In New Zealand, surveillance indicated that in 2006 the incidence rate of campylobacteriosis was 379.3 per 100,000 population (Pirie et al., 2009). A previous national case-control study had concluded that contact with raw or undercooked poultry meat and eating poultry in restaurants were major risks of campylobacteriosis with a PAF of >50% (Eberhart-Phillips et al., 1997). A national survey reported that over 80% of chicken meat samples at retail were *Campylobacter* contaminated while MLST data analysis indicated that between 52 and 75% of human infections were attributable to poultry (French and the Molecular Epidemiology and Veterinary Public Health Group, 2008). In response to a considerable media campaign NZFSA adopted a vigorous risk management strategy from 2006 onwards to reduce human cases by reducing poultry meat contamination. The strategy included public education via advertisements concentrating on hazards from cooking on barbecues.

From April 2007 all flocks were caecally sampled and cultured for prevalence of *Campylobacter* and a target for carcass contamination was established by agreement with industry. The industry also engaged in educating poultry farmers concerning biosecurity, developing leak-proof packaging and working with the regulators. On farm biosecurity improvements were made and at the production level hygiene was improved and hyperchlorinated water was used to cool birds post dressing. As a result of these measures the average prevalence of contaminated carcasses was reduced from 57% to 31% in the second quarters of 2007 and 2008 respectively. Similarly contamination levels (mean log count) were reduced from 3.07 to 2.41per carcass respectively[13]. Figure 4 shows the trend over the period 1997-2008 in the incidence rate of campylobacteriosis, which decreased to 156.8 per 100,000 population in 2008 (Williman et al., 2009). The modified Hald model (see Section 4.1.4.4.) has been extended to incorporate time (French et al., 2009) in a source attribution model for the Manawatu region of New Zealand. The work demonstrates that the reduction in incidence in human campylobacteriosis resulted in a smaller proportion of human cases being attributed to poultry (see Figure 5).

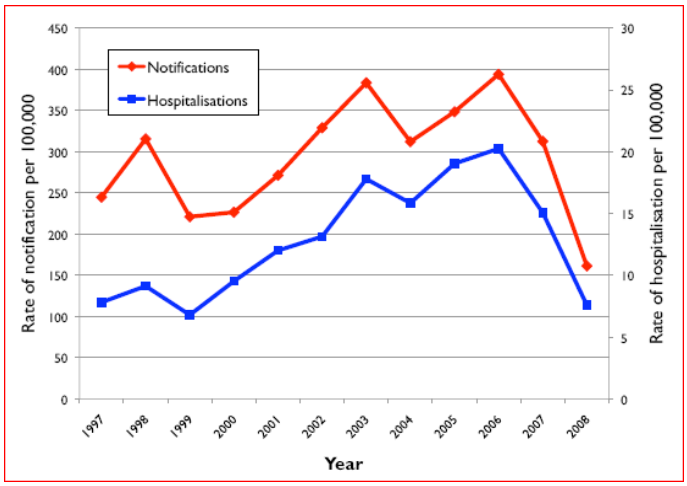

Figure 4: Rate of notification and hospitalisation per 100,000 population, 1997-2008, New Zealand[14].

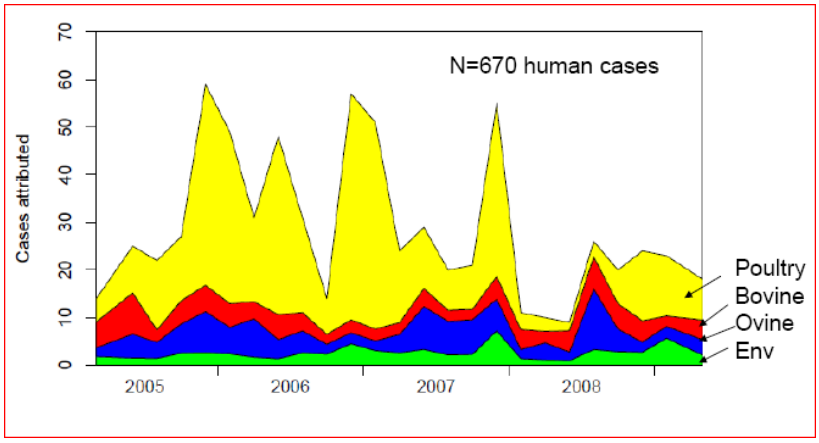

Figure 5: Dynamics of source attribution between 2005 and 2009 in the Manawatu region, New Zealand[15].

13 See http://www.nzfsa.govt.nz/publications/food-focus/2009-02/page-04.htm#TopOfPage
14 See http://www.nzfsa.govt.nz/science/workshops-presentations/28-october-09/11-workshop-validating-the-outcome.pdf

In Denmark, a voluntary intervention strategy for *Campylobacter* in broiler production was implemented in 2003. However, voluntary interventions relating to bio-security at the farm level were introduced as early as the late 1990s. The 2003 strategy comprised initiatives at all stages of the food production chain. This included, to the extent possible, the channelling, by two major slaughter companies (>98% of the Danish broiler production), of *Campylobacter*-positive flocks to freezing before retail, based on detection by culture 7-10 days before slaughter. The prevalence of positive flocks (as detected by polymerase chain reaction (PCR) on cloacal samples at slaughter) decreased from 43% in 2002 to 27% in 2007. Similarly the prevalence of positive meat samples decreased from 18% in 2004 to 8% in 2006-2007. Over the similar time period the reported cases of human campylobacteriosis in Denmark decreased by 12% (Rosenquist et al., 2009) This contemporaneous decrease in the number of reported human cases was considered due, at least in part, to a decrease in *Campylobacter* in broilers and broiler meat as a result of the intervention strategy implemented.

Two "natural experiments" involving poultry have been investigated for their effect on campylobacteriosis.

In 1999 dioxins were detected in poultry feed in Belgium, resulting in the withdrawal of all domestically produced poultry meat and eggs from the Belgian market (which represent around half of the market share in Belgium). A national reduction in campylobacteriosis cases of 40% was observed (Vellinga and Van Loock, 2002).

During March – beginning of May 2003, as a result of an outbreak of avian influenza, about 1,300 commercial and >17,000 non-commercial flocks (largely broilers and layers) were culled in a small (50x30Km) central region of the Netherlands (Van Pelt et al., 2009). This cull was associated with an immediate 40% reduction in cases of campylobacteriosis which declined to a reduction of 25% over the rest of 2003. The effect was most strong within the region affected by the cull. During this period there was a reduction in broiler meat sales but the overall reduction in cases was considered to only partly be attributable to reduced consumption of broiler meat. The data suggests that a reduction in indirect transmission routes as a result of decreased environmental contamination from poultry also had a significant effect.

7. Attribution through expert elicitation

The use of expert opinion as a methodology for source attribution of human food-borne illness was discussed in a recent EFSA opinion (EFSA, 2008a), from which part of the information reported below is extracted.

Expert opinion is used to fill data gaps or to combine the data from different studies and scientific approaches into a single estimate. Expert judgements are subjective by nature and may be biased by the specific background and scientific expertise of the respondents. Methods exist to evaluate the expert's performance. A structured procedure also helps to avoid many other pitfalls that may arise when asking experts for their subjective estimates. These structured approaches require more resources and technical expertise than conventional, unstructured evaluations and need a multidisciplinary approach. This may hamper their acceptance in practice. Expert estimates typically combine information from different sources, which can be considered both a strength and a weakness. There are currently no analytical approaches for combining data from diverse sources, e.g. outbreak studies and epidemiological studies of sporadic cases, but expert judgement can present a practical alternative source of information. However, the actual evaluation of the data and the weight put on any single data source lacks transparency.

15 www.nzfsa.govt.nz/science/workshops-presentations/28-october-09/8-workshop-microbial-subtyping-ans-sentinel-sites-in-nz.pdf

To date, a number of studies have been performed using expert elicitation to attribute source of food-borne disease. Three studies, from the USA (Hoffmann et al., 2007; Mead et al., 1999) and Australia (Hall et al., 2005) have estimated the proportion of food-borne transmission of campylobacteriosis as between 75 and 80%. An additional study, performed in the Netherlands, reported considerably lower estimates (42% food-borne transmission and 54% of food-borne cases due to chicken) (Havelaar et al., 2008). One explanation for this difference is that in the Dutch study experts were not only asked to estimate the proportion of food-borne cases but also the proportion attributable to direct contact with animals, the environment and human-to-human transmission.

8. Discussion

This section will compare and discuss the results of different attribution methods, while drawing conclusions relative to the strengths and weaknesses of the different methods used and taking into account data quality and availability.

8.1. Chicken and broiler meat as sources of human campylobacteriosis

The results from different attribution approaches, as applied in different countries, confirm previous epidemiological investigations that poultry is a major, if not the largest, single source of human infection. The proportion attributed by microbial subtyping to chicken as a reservoir ranges between 50 and 80%. In comparison, recent case-control studies suggest a lower proportion of 24-29% attributed to the consumption of chicken meat, which is similar to 29% of the outbreaks attributed to a known source being associated with chicken meat. The apparent discrepancy between the results of these different attribution methods could be associated with several factors. MLST studies may overestimate the importance of chicken due to incomplete data on other reservoirs, such as pets and wild birds. If more data on the diversity of strains in such reservoirs became available, a larger proportion of human cases might be linked to these reservoirs. Furthermore, strains from the chicken reservoir may reach humans by pathways other than via meat (e.g. via the environment or direct contact) and "poultry-associated" strains can also colonise other hosts such as cattle, pigs and pets. The possible role of indirect routes is underlined by the Dutch experience during the avian influenza crisis in 2003, when culling of predominantly laying hens resulted in a significant decrease of human campylobacteriosis, while the Belgian dioxin crisis in 1999 suggested a more important role for broiler meat. Conversely, case control studies may underestimate the true attributable risks due to inaccurate exposure assessment and to confounding by acquired immunity. Such inaccuracies typically bias the risk estimates to the null, and as a consequence case control studies usually do not explain all cases by identified risk factors. *Campylobacter* is an infrequent cause of reported outbreaks, and the available data from outbreak investigations is limited. Consequently, at this time, outbreak data is not considered to be the most appropriate approach for attributing human campylobacteriosis.

8.2. Strengths and weaknesses of attribution methods

Information on the most important advantages and drawbacks of the source attribution methods discussed in this opinion are summarized in Table 7. This Table is based on information included in a previous EFSA opinion (EFSA, 2008a) with specific details for attribution of campylobacteriosis added.

The Manawatu, New Zealand studies (French and the Molecular Epidemiology and Veterinary Public Health Group, 2008) indicate that a number of the attribution models for microbial subtyping (Dutch, modified Hald and Asymmetric island models) give similar results, as do the results from Scotland (Sheppard et al., 2009). However, it should be noted that this does not necessarily mean that they are correct, i.e. that the percentages attributable to each source are actually true. There is no explicit incorporation of exposure, survival or infectivity of the organisms. Furthermore, all of these models

depend on the suitability and the representativeness of MLST data and it can be problematic to obtain sufficient information on rare subtypes from humans and putative reservoirs. In New Zealand a number of *C. jejuni* isolates have been obtained from environmental sources which are unique and have no known host reservoir. It is likely that they belong to yet unidentified wildlife reservoir (Mullner et al., 2009b). However, the self attribution of strains does demonstrate that there are variations in the frequencies of the MLST sources that are found in the different reservoirs making attribution plausible.

The recent work by French et al. (2009) demonstrating the link between the reduction in incidence in human campylobacteriosis cases attributed to poultry and the interventions predominantly applied to the poultry industry, although preliminary, showed the power of the source attribution techniques for monitoring the effect of interventions.

Comparative exposure assessment currently has serious limitations in predicting the contribution of each source to the burden of human campylobacteriosis due to many data gaps, including the uncertainty of the events in the different possible pathways (e.g. cross-contamination in the kitchen) and the sparcity of knowledge of the dose-response relationship. At present this approach can be used primarily to stimulate the generation of hypotheses (EFSA, 2009c). It seems that developing a methodology which can link the subtyping-based models with comparative risk assessments, for each of the pathways through which humans might be infected by *Campylobacter,* is a potential future direction. In addition, the phenotypic properties of strains, such as virulence, colonisation and survival potentials, need to be incorporated. To this end, much research is still required on quantifying human exposure, infectivity of strains, survival of the organisms, etc. However, this may be difficult and expensive to achieve in the short term.

The systematic review (SR) of case-control studies presented in this opinion followed a rigorous search strategy to identify all potentially relevant peer-review case-control studies of sporadic campylobacteriosis. Collected studies were conducted in a wide variety of countries and time periods, designed with different settings, and sometimes focused on specific age groups within the population. The quality of the studies also varied, and was evaluated on the basis of defined methodological criteria during the formal process of the SR, and not judged on an individual basis by the reviewers. The risk factors extracted from individual studies were categorized by "main source-classification schemes", and the meta-analyses of collected data were undertaken using risk factor strata, analyzing information from all references that assessed the impact of that specific factor on the risk of disease. Such categorization implies the harmonization of risk factors or food labels, which may have resulted in loss of information from individual studies. Nonetheless, this approach enabled the integration and meta-analysis of results from all collected studies.

Estimates presented in this opinion demonstrate the relative importance of various risk factors for the overall population in the world or European regions, regardless of age, sex or country of citizenship, and for all campylobacters, regardless of species or subtype. However, the epidemiology of *Campylobacter* is varied, and different human populations, e.g. different age groups or populations from rural and urban areas may face different risks. Moreover, the immune status of individuals can affect epidemiological results. The meta-analysis of case-control studies investigated risk factors for children but did not allow for sub-analyses by population distribution or risk groups. Additionally, it has been demonstrated that differences in risk factors exist for different *Campylobacter* species, suggesting that the aggregation of these bacterial species, as done in most case-control studies, may mask important species-specific risk factors (Doorduyn et al., 2009; Gillespie et al., 2002).

Causes of outbreaks were found to differ substantially according to European region. For example, there were three reported outbreaks with drinking water as an implicated source, all in Northern European countries. These differences may reflect variations in the prevalence of the pathogens in investigated sources, in consumption habits in the different countries or in the data quality and availability. A recent analysis of outbreak data for source attribution in Canada suggested changes in

attribution to sources over time (Ravel et al., 2009), highlighting the importance of using recent data that does not aggregate information from many years. The use of spatial analysis methodologies for exploring associate risk factors (e.g. age distribution of human cases), or a combination of epidemiological studies with microbial subtyping and modelling techniques may in some situations help overcoming these limitations.

Table 7: Most important strengths and limitations of source attribution approaches.

	Strengths	Limitations
Microbiological approaches		
Microbial subtyping	- Might be able to identify the most important reservoirs of the zoonotic agent, assisting prioritization of where to focus control strategies at the animal level. - Attributes illness to the primary source (animal reservoir or retail products depending on sampling), reducing uncertainty due to cross-contamination and the risk of attribution to an "accidental" source. - Is able to follow trends over time. - Validation is possible using isolates from known sources.	- *Campylobacter* is weakly heterogeneously distributed among some important host reservoirs. - No information provided on the different pathways through which the pathogen can be transmitted to humans. - Data intensive, requiring a collection of representative isolates from all (major) sources and therefore resource demanding. - Subtypes can vary a lot between seasons, countries etc. but also throughout the food chain, which is not accounted for by some models. - Standardised subtyping methods are required (methods are changing over time).
Comparative exposure assessment	- Attributes illness to the point of exposure, taking into account the different transmission routes from the same reservoir. - Once a model is developed, new data can be easily included.	- Often limited by lack of sufficient data, which results in large uncertainties around the estimates. - There is no linear relation between exposure and illness.
Epidemiological approaches		
Case-control studies	- Valuable tool to identify relevant risk factors for human infections, including sources of exposure, predisposing, behavioural or seasonal factors. - A systematic review of published case-control studies can provide an overview of the relevant exposures and risk factors for that infection. - Can identify a wide range of familiar and unfamiliar risk factors.	- Misclassification due to immunity may reduce attributable risk or even suggest protection. - Most studies only explain a small fraction of all cases. - Cases may reflect a mixture of possible sources of exposure, and it may be difficult to distinguish between these exposures. - Statistical power to determine the importance of common exposures often requires enrolment of many participants. - Misclassification of exposures due to lack of accuracy of recall may lead to an underestimation of the burden of illness attributed to specific exposures. - Need for standardisation of exposure measurement through questionnaires (*Campylobacter* is highly seasonal and in many studies cases and controls are asked questions in different time points).

Analysis of data from outbreaks	- Clear documentation that a specific pathogen was transmitted to humans via a specific food item can be available. - Data may capture the effect of contamination at multiple points from the farm to consumption. - A wide variety of food vehicles are represented, including less frequently identified food items. - Data from outbreak investigations may, in some countries or regions, be the most readily available source of information for source attribution.	- Outbreaks may not be detected, investigated or reported. - Only a fraction of cases of human campylobacteriosis is associated with human outbreaks. - Quality of evidence varies and classification schemes for the data are not consistently used. - Large outbreaks, outbreaks associated with point sources, outbreaks that have short incubation periods, and outbreaks that cause serious illness are more likely to be investigated. - Illnesses included in data from outbreak investigations may not be representative of all food-borne illnesses. - Certain food vehicles are more likely to be associated with reported outbreaks than others, which can lead to an overestimation of the proportion of human illnesses attributed to a specific food.
Intervention studies		
	- Allows for a direct measure of the impact of a given source on the number of human cases of infection, avoiding the account for the effect of external sources or risk factors. - Large scale; reflecting all complexities across the food chain.	- Interpretation of data from "large-scale" interventions is difficult, since usually several interventions are implemented at the same time. - Complex and resource demanding studies. - Need for an extensive surveillance system in place. - Occurrence of natural experiments is unpredictable.
Expert elicitation		
	- Useful for providing interim results in countries where data are lacking. - Useful to evaluate the credibility of different attribution approaches.	- Conclusions are based on individual judgment, which may be misinformed or biased.

8.3. Data quality and availability

For all attribution methods employed, there was a lack of representative data for the different countries and regions of the EU. *Campylobacter* isolates are not systematically collected from humans or putative reservoirs in most MS, and typing of isolates (if at all) is frequently limited to species determination without subtyping. Countries in which subtyping has been applied use a variety of methods, while attribution models have only been developed for MLST. To obtain more representative data for the whole EU, it would be necessary to build representative collections of isolates in as many MS as possible, taking the regional differences in epidemiology of *Campylobacter* into account. Such collections should, as a minimum, be typed using MLST and both the isolates and their DNA should be stored for future use. Case-control studies have been carried out for several decades in many MS, but the majority are from North-Western Europe. Similar studies from new MS would provide a broader basis for attribution at a European level. New studies should take differences between *Campylobacter* species, age groups and other relevant factors into account. Food-borne outbreak investigations and reporting systems are not yet harmonised within the EU, and thus differences in numbers and types of reported outbreaks and the causative agents involved do not necessarily reflect different levels of food safety between MS. However, some limitations may be overcome with the use of data from European countries for multiple years, i.e. from 2007 onwards, based on the new system for reporting outbreaks within the EU. While natural experiments may provide a useful insight into the relevant importance of particular risk factors, their occurrence is unpredictable and their interpretation is difficult due to incomplete information on potential confounders. Nevertheless, such opportunities should be exploited when they occur and principles developed to ensure that appropriate studies are quickly implemented. The same principles should be applied to evaluation of interventions on a national scale, as usually many factors change simultaneously and it is difficult to attribute a change in disease incidence to one particular factor. A recent EFSA Colloquium has recommended that "*The assessment of management interventions should ideally be carried out in the framework of controlled studies. […] Intervention studies should ideally be carried out in pilot areas and with proper controls before the extension to a broader scale (e.g., at the national level). If this is feasible, it will permit to properly assess the impact of possible mitigation actions on a small scale, before their more general application*" (EFSA, 2009c).

There is a particular need to better understand the role of acquired immunity on the epidemiology of human campylobacteriosis and its impact on both attribution and risk assessment studies. As mentioned earlier in this document, it has recently been reported that most infections with *Campylobacter* are asymptomatic, but still give rise to a detectable immune response (Havelaar et al., 2009). How far these immune responses are indicative of protective immunity remains unknown but there is considerable evidence to support the hypothesis that acquired protective immunity is an important factor, not only for highly exposed individuals (e.g. in developing countries or during occupational exposure) but also for less exposed citizens of industrialised countries. The effect of immune protection may result in misclassification of exposure in case-control studies, since controls that have been exposed may not become ill. Immunity among controls may also explain the fact that most case-control studies cannot account for the majority of the cases (Swift and Hunter, 2004). Therefore, there is a need to develop surrogate measures of protective immunity rather than just antibody detection in order to quantify the prevalence and dynamics of susceptibility to infection, to better understand the degree of cross-protection conferred by repeated exposure, especially to other subtypes, and to develop methods to incorporate the effects of acquired immunity in attribution and risk assessment models.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Answer to the terms of reference:

- Handling, preparation and consumption of broiler meat may account for 20% to 30% of human cases of campylobacteriosis, while 50% to 80% may be attributed to the chicken reservoir as a whole. These estimates are based on the following data:
    - Available subtyping studies using multi-locus sequence typing (MLST) and probabilistic models from three geographical areas suggest that between 50 and 80% of all human cases of *C. jejuni* can be attributed to chickens as a reservoir.
    - A meta-analysis of case-control studies suggests a variety of risk factors including travelling, animal contact, food and untreated drinking water. Among food sources, eating chicken or poultry (in particular when undercooked or in restaurants) is an important risk factor. Recent studies have estimated that between 24-29% of human cases can be attributed to handling, preparation and consumption of chicken meat.
    - Even though most cases of human campylobacteriosis are sporadic, outbreaks are reported in the EU. Outbreak isolates were rarely identified to the species level. Recent studies suggested that over one quarter of the outbreaks for which a source was identified can be attributed to chicken. For two thirds of the outbreaks the source was unknown.
    - "Natural experiments" in which the consumption of chicken meat was temporarily reduced due to crises in the food chain (dioxin contamination of chicken meat in Belgium and avian influenza in the Netherlands) supported the importance of chicken as a major reservoir of human infections and chicken meat as an important pathway. Interventions in Iceland and New Zealand to reduce consumer exposure to highly contaminated chicken meat were accompanied by marked reductions in reported campylobacteriosis cases.
- The lower proportion of human cases related to chicken meat identified by case-control studies as compared to microbial subtyping may be explained by several factors. Case control studies attribute at the point of exposure, while subtyping studies attribute at reservoir level. Case control studies may underestimate the true attributable risks due to inaccurate exposure assessment and to confounding by acquired immunity. MLST studies may overestimate the importance of chicken due to incomplete data on reservoirs other than farm animals. Strains from the chicken reservoir may reach humans by pathways other than food (e.g. by the environment or by direct contact).
- Data for source attribution in the EU are limited and unavailable for the majority of Member States (MS) and there are indications that the epidemiology of human campylobacteriosis differs between regions. Hence, these conclusions must be interpreted with care.

General conclusions:

- The principal reservoirs of *Campylobacter* spp. are the alimentary tracts of wild and domesticated birds and mammals. There are multiple pathways of human exposure.
- Campylobacteriosis is now the most frequently reported zoonotic illness in the EU. There is considerable underascertainment and underreporting, and the true number of cases of illness is likely to be 10-100 times higher than the reported number. Serosurveillance indicates that European citizens may be exposed to *Campylobacter* sufficiently to produce an immune response every 1-3 years. There may be not less than 2 million and possibly as high as 20 million cases of clinical campylobacteriosis per year in the EU27.

- *Campylobacter jejuni* is the species most frequently isolated from human campylobacteriosis cases throughout the EU, followed by *C. coli*. The main livestock reservoirs of *C. jejuni* are poultry and ruminants, whereas *C. coli* is mainly found in pigs and poultry.

- Travelling outside the country of residence is a reported risk factor for human campylobacteriosis. A large proportion of cases is associated with travelling within the EU and would be preventable by EU-wide control measures.

- Source attribution based on microbial subtyping has only recently been applied to campylobacteriosis. Recently, sequence-based genotyping methods, and in particular MLST, have increasingly been used to elucidate the population structure of these bacteria.

- All subtyping techniques have shown the population structure of *C. jejuni* to be highly diverse and weakly clonal. There are multiple subtypes, many of which are rare. The subtype distribution may differ between seasons, geographic locations or even over the years. Some subtypes have a statistical association with specific reservoirs and there is considerable overlap between subtypes from cases of human disease and subtypes from farm animals, in particular from chicken.

- Current subtyping techniques do not provide any indication of strain virulence. The mechanisms of disease caused by *Campylobacter* are still poorly understood and it is not yet possible to relate differences in genotypes and phenotypes to virulence.

- Most isolates for which subtyping data are available are not based on representative sampling. Non-human isolates mainly originate from poultry and ruminants. There is less data on other reservoirs, including pets and wild birds.

- Case-control studies suggest that risk factors depend on the species of *Campylobacter*. For example, recreational water was strongly associated with *C. coli* in one study. Furthermore, risk factors and/or their quantitative importance may depend on the age of human cases.

RECOMMENDATIONS

- To estimate the burden of disease and to give a baseline for allowing the evaluation of the human health effects of any interventions, it is recommended to establish active surveillance of campylobacteriosis in all MS, including efforts to quantify the level of underascertainment and underreporting.

- To provide a better understanding of the molecular epidemiology of campylobacteriosis and a better basis for source attribution in the EU, it is recommended that a representative collection of isolates from humans and putative reservoirs (in particular food-producing animals but also other domestic and wild animals) is obtained and subjected to genotyping in all MS. Currently, the most appropriate method of genotyping appears to be MLST. It is recommended to store the isolates and their DNA so that, in the future, potentially improved subtyping methods could be applied.

- It is recommended that research is undertaken to identify markers of *Campylobacter* virulence and survival properties, as such information would be generally useful for risk assessment, including for the improvement of the resolution of future attribution studies.

- It is recommended that future studies on risk factors for campylobacteriosis take into account differences in the ecology of *Campylobacter* species, and subtypes within species.

- Further studies are recommended to develop methodologies to quantify the impact of acquired protective immunity on the epidemiology of campylobacteriosis in the EU, which will be the basis to develop more accurate models for source attribution based on case-control studies.

REFERENCES

Abuoun M, Manning G, Cawthraw SA, Ridley A, Ahmed IH, Wassenaar TM and Newell DG, 2005. Cytolethal distending toxin (CDT)-negative *Campylobacter jejuni* strains and anti-CDT neutralizing antibodies are induced during human infection but not during colonization in chickens. Infect Immun, 73, 3053-62.

Adak GK, Meakins SM, Yip H, Lopman BA and O'Brien SJ, 2005. Disease risks from foods, England and Wales, 1996-2000. Emerg Infect Dis, 11, 365-72.

Ang CW, Krogfelt K, Herbrink P, Keijser J, van Pelt W, Dalby T, Kuijf M, Jacobs BC, Bergman MP, Schiellerup P and Visser CE, 2007. Validation of an ELISA for the diagnosis of recent *Campylobacter* infections in Guillain-Barre and reactive arthritis patients. Clin Microbiol Infect, 13, 915-22.

Batz MB, Doyle MP, Morris G, Jr., Painter J, Singh R, Tauxe RV, Taylor MR and Wong DM, 2005. Attributing illness to food. Emerg Infect Dis, 11, 993-9.

Broman T, Palmgren H, Bergstrom S, Sellin M, Waldenstrom J, Danielsson-Tham ML and Olsen B, 2002. *Campylobacter jejuni* in black-headed gulls (*Larus ridibundus*): prevalence, genotypes, and influence on *C. jejuni* epidemiology. J Clin Microbiol, 40, 4594-602.

Broman T, Waldenstrom J, Dahlgren D, Carlsson I, Eliasson I and Olsen B, 2004. Diversities and similarities in PFGE profiles of *Campylobacter jejuni* isolated from migrating birds and humans. J Appl Microbiol, 96, 834-43.

Callicott KA, Hargardottir H, Georgsson F, Reiersen J, Frigriksdottir V, Gunnarsson E, Michel P, Bisaillon JR, Kristinsson KG, Briem H, Hiett KL, Needleman DS and Stern NJ, 2008. Broiler *Campylobacter* contamination and human campylobacteriosis in Iceland. Appl Environ Microbiol, 74, 6483-94.

Carrique-Mas J, Andersson Y, Hjertqvist M, Svensson A, Torner A and Giesecke J, 2005. Risk factors for domestic sporadic campylobacteriosis among young children in Sweden. Scand J Infect Dis, 37, 101-10.

Champion OL, Gaunt MW, Gundogdu O, Elmi A, Witney AA, Hinds J, Dorrell N and Wren BW, 2005. Comparative phylogenomics of the food-borne pathogen *Campylobacter jejuni* reveals genetic markers predictive of infection source. Proc Natl Acad Sci U S A, 102, 16043-8.

Charlett A, Cowden JM, Frost JA, Gillespie IA, Millward J, Neal KR, O'Brien JSJ, Painter MJ, Syed Q and Tompkins D, 2002. Ciprofloxacin resistance in *Campylobacter jejuni*: case-case analysis as a tool for elucidating risks at home and abroad. Journal of Antimicrobial Chemotherapy, 50, 561-568.

Charlett A, Cowden JM, Frost JA, Gillespie IA, Millward J, Neal KR, O'Brien SJ, Painter MJ, Syed Q and Tompkins D, 2003. Foreign and domestic travel and the risk of *Campylobacter* infection: Results from a population-based sentinel surveillance scheme. Journal of Travel Medicine, 10, 136-138.

Clayton D and Hills M, 1993. Statistical models in epidemiology. Oxford University Press, USA.

De Melker HE and Conyn-van Spaendonck MA, 1998. Immunosurveillance and evaluation of national immunization programmes: a population-based approach. Epidemiol. Infect., 121, 637-43.

Dekeyser P, Gossuin-Detrain M, Butzler JP and Sternon J, 1972. Acute enteritis due to related vibrio: first positive stool cultures. J Infect Dis, 125, 390-2.

Devane ML, Nicol C, Ball A, Klena JD, Scholes P, Hudson JA, Baker MG, Gilpin BJ, Garrett N and Savill MG, 2005. The occurrence of *Campylobacter* subtypes in environmental reservoirs and potential transmission routes. J Appl Microbiol, 98, 980-90.

Dingle KE, Colles FM, Ure R, Wagenaar JA, Duim B, Bolton FJ, Fox AJ, Wareing DR and Maiden MC, 2002. Molecular characterization of *Campylobacter jejuni* clones: a basis for epidemiologic investigation. Emerg Infect Dis, 8, 949-55.

Dingle KE, Colles FM, Wareing DR, Ure R, Fox AJ, Bolton FE, Bootsma HJ, Willems RJ, Urwin R and Maiden MC, 2001. Multilocus sequence typing system for *Campylobacter jejuni*. J Clin Microbiol, 39, 14-23.

Domingues AR, Pires SM, Lundsby K and Hald T, 2009. Source attribution of human salmonellosis and campylobacteriosis using a systematic review of studies of sporadic infections. Med-Vet-Net 5th Annual Scientific Meeting. 3-6 June 2009, El Escorial, Madrid, Spain.

Doorduyn Y, Breukink BJ, van der Brandhof WE, van Duynhoven YTHP, Wagenaar JA and van Pelt W, 2009. Case-case comparison of *Campylobacter coli* and *jejuni* infections and age-, season- and urbanization-specific risk factors for *Campylobacter jejuni* infections. Med-Vet-Net 5th Annual Scientific Meeting, 3-6 June 2009. El Escorial, Madrid, Spain.

Doorduyn Y, Van Pelt W, Siezen CL, Van Der Horst F, Van Duynhoven YT, Hoebee B and Janssen R, 2008. Novel insight in the association between salmonellosis or campylobacteriosis and chronic illness, and the role of host genetics in susceptibility to these diseases. Epidemiol Infect, 136, 1225-34.

Duim B, Wassenaar TM, Rigter A and Wagenaar J, 1999. High-resolution genotyping of *Campylobacter* strains isolated from poultry and humans with amplified fragment length polymorphism fingerprinting. Appl Environ Microbiol, 65, 2369-75.

Eberhart-Phillips J, Walker N, Garrett N, Bell D, Sinclair D, Rainger W and Bates M, 1997. Campylobacteriosis in New Zealand: results of a case-control study. J Epidemiol Community Health, 51, 686-91.

EFSA, 2005a. The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Antimicrobial Resistance in the European Union in 2004. The EFSA Journal, 310.

EFSA, 2005b. Opinion of the Scientific Panel on Biological Hazards on *Campylobacter* in animals and foodstuffs. The EFSA Journal, 173, 1-10.

EFSA, 2006. The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union in 2005. The EFSA Journal, 94.

EFSA, 2007. The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union in 2006. The EFSA Journal, 130.

EFSA, 2008a. Scientific Opinion of the Panel on Biological Hazards on a request from EFSA on Overview of methods for source attribution for human illness from food borne microbiological hazards. The EFSA Journal, 764, 1-43.

EFSA, 2008b. Scientific Opinion of the Panel on Biological Hazards on a request from the European Commission on a quantitative microbiological risk assessment on *Salmonella* in meat: Source attribution for human salmonellosis from meat. The EFSA Journal, 625, 1-32.

EFSA, 2009a. The Community Summary Report on Food-borne Outbreaks in the European Union in 2007. The EFSA Journal, 271.

EFSA, 2009b. The Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union in 2007. The EFSA Journal, 223.

EFSA 2009c. EFSA Scientific Colloquium Summary Report - Assessing health benefits of controlling *Campylobacter* in the food chain. 4-5 December 2008, Rome, Italy.

EFSA, 2009d. Joint Opinion on antiimicrobial resistance (AMR) focused on zoonotic infections. Scientific Opinion of the European Centre for Disease Prevention and Control; Scientific Opinion of the Panel on Biological Hazards; Opinion of the Committee for Medicinal Products for Veterinary Use; Scientific Opinion of the Scientific Committee on Emerging and Newly Identified Health Risks. The EFSA Journal, 7 (11) 1372.

EFSA, 2009e. Report on the availability of molecular typing methods for *Salmonella*, *Campylobacter*, verotoxigenic *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus* isolates from food, animals and feedingstuffs in European Union Member States (and in some other reporting countries). The EFSA Journal, 272, 1-52.

EFSA, 2009f. Scientific Opinion of the Panel on Biological Hazards on a request from European Commission on Quantitative estimation of the impact of setting a new target for the reduction of *Salmonella* in breeding hens of *Gallus gallus*. The EFSA Journal, 1036, 1-68.

Ekdahl K and Andersson Y, 2004. Regional risks and seasonality in travel-associated campylobacteriosis. BMC Infect Dis, 4, 54.

Endtz HP, Ruijs GJ, van Klingeren B, Jansen WH, van der Reyden T and Mouton RP, 1991. Quinolone resistance in *Campylobacter* isolated from man and poultry following the introduction of fluoroquinolones in veterinary medicine. J Antimicrob Chemother, 27, 199-208.

Engberg J, 2006. Contributions to the epidemiology of *Campylobacter* infections. A review of clinical and microbiological studies. Dan Med Bull, 53, 361-89.

Ethelberg S, Olsen KE, Gerner-Smidt P and Molbak K, 2004. Household outbreaks among culture-confirmed cases of bacterial gastrointestinal disease. Am J Epidemiol, 159, 406-12.

Ethelberg S, Simonsen J, Gerner-Smidt P, Olsen KE and Molbak K, 2005. Spatial distribution and registry-based case-control analysis of *Campylobacter* infections in Denmark, 1991-2001. Am J Epidemiol, 162, 1008-15.

Evers EG, Van Der Fels-Klerx HJ, Nauta MJ, Schijven JF and Havelaar AH, 2008. *Campylobacter* source attribution by exposure assessment. Int J Risk Ass Mgt, 8, 174–190.

Falkenhorst G, 2009. Sero-incidence of human infections with *Salmonella* and *Campylobacter* in Europe: comparison with incidence of reported cases and prevalence in food animals. Med-Vet-Net 5th Annual Scientific Meeting. 3-6 June 2009, El Escorial, Madrid, Spain.

FDA 2001. The Human Health Impact of Fluoroquinolone Resistant *Campylobacter* Attributed to the Consumption of Chicken. October 18, 2000, revised January 5, 2001.

Fearnley C, Manning G, Bagnall M, Javed MA, Wassenaar TM and Newell DG, 2008. Identification of hyperinvasive *Campylobacter jejuni* strains isolated from poultry and human clinical sources. J Med Microbiol, 57, 570-80.

Forbes KJ, Gormley FJ, Dallas JF, Labovitiadi O, MacRae M, Owen RJ, Richardson J, Strachan NJ, Cowden JM, Ogden ID and McGuigan CC, 2009. *Campylobacter* immunity and coinfection following a large outbreak in a farming community. J Clin Microbiol, 47, 111-6.

French N, Barrigas M, Brown P, Ribiero P, Williams N, Leatherbarrow H, Birtles R, Bolton E, Fearnhead P and Fox A, 2005. Spatial epidemiology and natural population structure of *Campylobacter jejuni* colonizing a farmland ecosystem. Environ Microbiol, 7, 1116-26.

French NP, Mullner P, Spencer SE, Wilson DJ, Jones G, Noble AD, Midwinter AC, Collins-Emerson JM, Campbell D, Hathaway S and Carter P, 2009. Controlling campylobacteriosis in New Zealand: the role played by molecular epidemiology and modelling. 15th International Workshop on *Campylobacter*, *Helycobacter* and related organisms. 2-5 September 2009, Niigata, Japan.

French NP and the Molecular Epidemiology and Veterinary Public Health Group HRI, 2008. Enhancing Surveillance of Potentially Foodborne Enteric Diseases in New Zealand: Human Campylobacteriosis in the Manawatu. Final report: FDI / 236 /2005.

Friedman CR, Hoekstra RM, Samuel M, Marcus R, Bender J, Shiferaw B, Reddy S, Ahuja SD, Helfrick DL, Hardnett F, Carter M, Anderson B and Tauxe RV, 2004. Risk factors for sporadic *Campylobacter* infection in the United States: A case-control study in FoodNet sites. Clin Infect Dis, 38 Suppl 3, S285-96.

Frost JA, Gillespie IA and O'Brien SJ, 2002. Public health implications of *Campylobacter* outbreaks in England and Wales, 1995-9: epidemiological and microbiological investigations. Epidemiol Infect, 128, 111-8.

Frost JA, Oza AN, Thwaites RT and Rowe B, 1998. Serotyping scheme for *Campylobacter jejuni* and *Campylobacter coli* based on direct agglutination of heat-stable antigens. J Clin Microbiol, 36, 335-9.

Fullerton KE, Ingram LA, Jones TF, Anderson BJ, McCarthy PV, Hurd S, Shiferaw B, Vugia D, Haubert N, Hayes T, Wedel S, Scallan E, Henao O and Angulo FJ, 2007. Sporadic *Campylobacter* infection in infants: a population-based surveillance case-control study. Pediatr Infect Dis J, 26, 19-24.

Garrett N, Devane ML, Hudson JA, Nicol C, Ball A, Klena JD, Scholes P, Baker MG, Gilpin BJ and Savill MG, 2007. Statistical comparison of *Campylobacter jejuni* subtypes from human cases and environmental sources. J Appl Microbiol, 103, 2113-21.

Gellynck X, Messens W, Halet D, Grijspeerdt K, Hartnett E and Viaene J, 2008. Economics of reducing *Campylobacter* at different levels within the Belgian poultry meat chain. J Food Prot, 71, 479-85.

Gerner-Smidt P, Hise K, Kincaid J, Hunter S, Rolando S, Hyytia-Trees E, Ribot EM and Swaminathan B, 2006. PulseNet USA: a five-year update. Foodborne Pathog Dis, 3, 9-19.

Gillespie IA, 2003. Ethnicity and *Campylobacter* infection: a population-based questionnaire survey. Journal of Infection, 47, 210-216.

Gillespie IA, O'Brien SJ, Frost JA, Adak GK, Horby P, Swan AV, Painter MJ and Neal KR, 2002. A case-case comparison of *Campylobacter coli* and *Campylobacter jejuni* infection: a tool for generating hypotheses. Emerg Infect Dis, 8, 937-42.

Gillespie IA, O'Brien S and Bolton FJ, 2009. Age Patterns of Persons with Campylobacteriosis, England and Wales, 1990-2007. Emerging Infectious Diseases, 15, 2046-48.

Gradel KO, Nielsen HL, Schonheyder HC, Ejlertsen T, Kristensen B and Nielsen H, 2009. Increased Short- and Long-Term Risk of Inflammatory Bowel Disease After *Salmonella* or *Campylobacter* Gastroenteritis. Gastroenterology, 137, 495-501.

Greig JD and Ravel A, 2009. Analysis of foodborne outbreak data reported internationally for source attribution. Int J Food Microbiol, 130, 77-87.

Gressmann H, Linz B, Ghai R, Pleissner KP, Schlapbach R, Yamaoka Y, Kraft C, Suerbaum S, Meyer TF and Achtman M, 2005. Gain and Loss of Multiple Genes During the Evolution of *Helicobacter pylori*. PLoS Genet, 1, e43.

Haagsma JA, Siersema PD, de Wit NJ and Havelaar AH, submitted for publication. Disease burden of post-infectious irritable bowel syndrome in the Netherlands.

Hald T, Vose D, Wegener HC and Koupeev T, 2004. A Bayesian approach to quantify the contribution of animal-food sources to human salmonellosis. Risk Anal, 24, 255-69.

Hall G, Kirk MD, Becker N, Gregory JE, Unicomb L, Millard G, Stafford R and Lalor K, 2005. Estimating foodborne gastroenteritis, Australia. Emerging Infectious Diseases, 11, 1257-1264.

Hanninen ML, Hakkinen M and Rautelin H, 1999. Stability of related human and chicken *Campylobacter jejuni* genotypes after passage through chick intestine studied by pulsed-field gel electrophoresis. Appl Environ Microbiol, 65, 2272-5.

Havelaar AH, Braunig J, Christiansen K, Cornu M, Hald T, Mangen MJ, Molbak K, Pielaat A, Snary E, Van Pelt W, Velthuis A and Wahlstrom H, 2007. Towards an integrated approach in supporting microbiological food safety decisions. Zoonoses Public Health, 54, 103-17.

Havelaar AH, de Wit MA, van Koningsveld R and van Kempen E, 2000. Health burden in the Netherlands due to infection with thermophilic *Campylobacter* spp. Epidemiol Infect, 125, 505-22.

Havelaar AH, Galindo AV, Kurowicka D and Cooke RM, 2008. Attribution of foodborne pathogens using structured expert elicitation. Foodborne Pathogens and Disease, 5, 649-659.

Havelaar AH, van Pelt W, Ang CW, Wagenaar JA, van Putten JPM, Gross U and Newell DG, 2009. Immunity to *Campylobacter*: its role in risk assessment and epidemiology. Critical Reviews in Microbiology, 35, 1-22.

Helms M, Simonsen J, Olsen KE and Molbak K, 2005. Adverse health events associated with antimicrobial drug resistance in *Campylobacter* species: a registry-based cohort study. J Infect Dis, 191, 1050-5.

Helms M, Vastrup P, Gerner-Smidt P and Molbak K, 2003. Short and long term mortality associated with foodborne bacterial gastrointestinal infections: registry based study. BMJ, 326, 357.

Hoffmann S, Fischbeck P, Krupnick A and McWilliams M, 2007. Using expert elicitation to link foodborne illnesses in the United States to foods. Journal of Food Protection, 70, 1220-1229.

Hofshagen M and Brunheim T, 2006. The surveillance and control programme for *Campylobacter* in broiler flocks in Norway. In: Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2005. HH Brun E, Mørk T, Jordsmyr HM (editors). Oslo: National Veterinary Institute, 119-123.

Hofshagen M and Kruse H, 2005. Reduction in flock prevalence of *Campylobacter* spp. in broilers in Norway after implementation of an action plan. J Food Prot, 68, 2220-3.

Hofshagen M and Opheim M, 2007. The surveillance and control programme for *Campylobacter* in broiler flocks in Norway. In: Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2006. HH Brun E, Mørk T, Jordsmyr HM (editors). Oslo: National Veterinary Institute, 127-132.

Hofshagen M and Opheim M, 2008. The surveillance and control programme for *Campylobacter* in broiler flocks in Norway. In: Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual report 2007. JH Brun E, Hellberg H, Mørk T (editors). Oslo: National Veterinary Institute, 131-134.

Hopkins KL, Desai M, Frost JA, Stanley J and Logan JM, 2004. Fluorescent amplified fragment length polymorphism genotyping of *Campylobacter jejuni* and *Campylobacter coli* strains and its relationship with host specificity, serotyping, and phage typing. J Clin Microbiol, 42, 229-35.

Jacobs BC, van Belkam A and Edntz HP, 2008. Guillain-Barre Syndrome and *Campylobacter* infection. In *Campylobacter* 3rd Ed. . In: I Nachamkin, Szymanski, C. and Blaser, M. ASM Press, Washington DC, 245-261.

Janssen R, Krogfelt KA, Cawthraw SA, van Pelt W, Wagenaar JA and Owen RJ, 2008. Host-pathogen interactions in *Campylobacter* infections: the host perspective. Clin Microbiol Rev, 21, 505-18.

Jolley KA, Chan MS and Maiden MC, 2004. mlstdbNet - distributed multi-locus sequence typing (MLST) databases. BMC Bioinformatics, 5, 86.

Jore S, Viljugrein H, Brun E, Heier BT, Borck B, Ethelberg S, Hakkinen M, Kuusi M, Reiersen J, Hansson I, Olsson Engvall E, Løfdahl M, Wagenaar JA, van Pelt W and Hofshagen M, 2009. Trends in *Campylobacter* incidence in broilers and humans in six European countries, 1997-2007. Preventive Veterinary Medicine, In Press.

Kapperud G, Espeland G, Wahl E, Walde A, Herikstad H, Gustavsen S, Tveit I, Natas O, Bevanger L and Digranes A, 2003. Factors associated with increased and decreased risk of *Campylobacter* infection: a prospective case-control study in Norway. Am J Epidemiol, 158, 234-42.

Karenlampi R, Rautelin H and Hanninen ML, 2007. Evaluation of genetic markers and molecular typing methods for prediction of sources of *Campylobacter jejuni* and *C. coli* infections. Appl Environ Microbiol, 73, 1683-5.

Kassenborg HD, Smith KE, Vugia DJ, Rabatsky-Ehr T, Bates MR, Carter MA, Dumas NB, Cassidy MP, Marano N, Tauxe RV and Angulo FJ, 2004. Fluoroquinolone-resistant *Campylobacter* infections: eating poultry outside of the home and foreign travel are risk factors. Clin Infect Dis, 38 Suppl 3, S279-84.

King EO, 1957. Human infections with *Vibrio fetus* and a closely related *Vibrio*. Journal of Infectious Diseases, 101, 119-128.

King EO, 1962. Laboratory recognition of *Vibrio fetus* and a closely related *Vibrio* isolated from cases of human vibriosis. Annals of the New York Academy of Sciences, 98, 700-&.

Kovats RS, Edwards SJ, Charron D, Cowden J, D'Souza RM, Ebi KL, Gauci C, Gerner-Smidt P, Hajat S, Hales S, Pezzi GH, Kriz B, Kutsar K, McKeown P, Mellou K, Menne B, O'Brien S, van Pelt W and Schmid H, 2005. Climate variability and *Campylobacter* infection: an international study. International Journal of Biometeorology, 49, 207-214.

Kramer JM, Frost JA, Bolton FJ and Wareing DR, 2000. *Campylobacter* contamination of raw meat and poultry at retail sale: identification of multiple types and comparison with isolates from human infection. J Food Prot, 63, 1654-9.

Lake R, Hudson A, Cressey P and Bayne G 2006. Quantitative risk mode: *Campylobacter* spp. in the poultry food chain. Report of the Institute of Environmental Science and Research Limited, Christchurch, New Zealand.

Luber P and Bartelt E, 2007. Enumeration of *Campylobacter* spp. on the surface and within chicken breast fillets. J Appl Microbiol, 102, 313-8.

Maiden MC, 2006. Multilocus sequence typing of bacteria. Annu Rev Microbiol, 60, 561-88.

Maiden MC, Bygraves JA, Feil E, Morelli G, Russell JE, Urwin R, Zhang Q, Zhou J, Zurth K, Caugant DA, Feavers IM, Achtman M and Spratt BG, 1998. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc Natl Acad Sci USA, 95, 3140-5.

Mangen MJJ, Havelaar AH, Bernsen RAJAM, Koningsveld R van and Wit GA de, 2005. The costs of human *Campylobacter* infections and sequelae in the Netherlands: a DALY and cost-of-illness approach. Acta Agricultura Scandinavica. Section C, Food Economics, 2, 35-51.

Manning G, Dowson CG, Bagnall MC, Ahmed IH, West M and Newell DG, 2003. Multilocus sequence typing for comparison of veterinary and human isolates of *Campylobacter jejuni*. Appl Environ Microbiol, 69, 6370-9.

McBride G, Meleason M, Skelly C, Lake R, van der Logt P and Collins R 2005. Preliminary relative risk assessment for *Campylobacter* exposure in New Zealand: 1. National model for four potential human exposure routes; 2. Farm environmental model. NIWA Client Report: HAM2005-094.

McCarthy ND, Colles FM, Dingle KE, Bagnall MC, Manning G, Maiden MC and Falush D, 2007. Host-associated genetic import in *Campylobacter jejuni*. Emerg Infect Dis, 13, 267-72.

McFadyean J and Stockman S, 1913. Report of the Departmental Committee appointed by the Board of Agriculture and Fisheries to inquire into epizootic abortion. Part III. Abortion in sheep. London: HMSO.

Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM and Tauxe RV, 1999. Food-related illness and death in the United States. Emerging Infectious Diseases, 5, 607-625.

Milnes AS, Stewart I, Clifton-Hadley FA, Davies RH, Newell DG, Sayers AR, Cheasty T, Cassar C, Ridley A, Cook AJC, Evans SJ, Teale CJ, Smith RP, McNnally A, Toszeghy M, Futter R, Kay A and Paiba GA, 2008. Intestinal carriage of verocytotoxigenic *Escherichia coli* O157, *Salmonella*, thermophilic *Campylobacter* and *Yersinia enterocolitica*, in cattle, sheep and pigs at slaughter in Great Britain during 2003. Epidemiology and Infection, 136, 739-751.

Molbak K and Havelaar AH, 2008. Burden of illness of campylobacteriosis and sequelae. In: *Campylobacter*, 3rd edition. Nachamkin I, Szymanski G and Blaser M (editors). ASM Press, Washington DC, 151-162.

Mullner P, 2009. Estimating the contribution of different sources to the burden of human campylobacteriosis and salmonellosis. PhD thesis, Massey University, New Zealand.

Mullner P, Jones G, Noble A, Spencer SEF, Hathaway S and French NP, 2009a. Source Attribution of Food-Borne Zoonoses in New Zealand: A Modified Hald Model. Risk Analysis, 29, 970-984.

Mullner P, Spencer SEF, Wilson DJ, Jones G, Noble AD, Midwinter AC, Collins-Emerson JM, Carter P, Hathaway S and French N, 2009b. Assigning the source of human campylobacteriosis in New Zealand: A comparative genetic and epidemiological approach. Infection, Genetics and Evolution, 9, 1311-19.

Nachamkin I, Engberg J and Møller Aarestrup F, 2000. Diagnosis and Antimicrobial Susceptibility of *Campylobacter* spp. In: *Campylobacter* 2nd edition. Nachamkin I and Blaser MJ (editors). ASM, Washington D.C., 45-66.

Nachamkin I, Ung H and Li M, 2002. Increasing fluoroquinolone resistance in *Campylobacter jejuni*, Pennsylvania, USA,1982-2001. Emerg Infect Dis, 8, 1501-3.

Neimann J, Engberg J, Molbak K and Wegener HC, 2003. A case-control study of risk factors for sporadic *Campylobacter* infections in Denmark. Epidemiol Infect, 130, 353-66.

Newell DG, 1982. *Campylobacter*. Epidemiology, pathogenesis and biochemistry. Proceedings of an International Workshop on *Campylobacter* Infections held. 24th-26th March 1981, University of Reading, UK.

Newell DG, 2001. Animal models of *Campylobacter jejuni* colonization and disease and the lessons to be learned from similar Helicobacter pylori models. Symp Ser Soc Appl Microbiol, 57S-67S.

Nichols GL, 2005. Fly transmission of *Campylobacter*. Emerg Infect Dis, 11, 361-4.

Norstrom M, Hofshagen M, Stavnes T, Schau J, Lassen J and Kruse H, 2006. Antimicrobial resistance in *Campylobacter jejuni* from humans and broilers in Norway. Epidemiol Infect, 134, 127-30.

Nylen G, Dunstan F, Palmer SR, Andersson Y, Bager F, Cowden J, Feierl G, Galloway Y, Kapperud G, Megraud F, Molbak K, Petersen LR and Ruutu P, 2002. The seasonal distribution of *Campylobacter* infection in nine European countries and New Zealand. Epidemiol Infect, 128, 383-90.

O'Brien SJ, Gillespie IA, Sivanesan MA, Elson R, Hughes C and Adak GK, 2006. Publication bias in foodborne outbreaks of infectious intestinal disease and its implications for evidence-based food policy. England and Wales 1992-2003. Epidemiol Infect, 134, 667-74.

O'Neill B, Ejidokun OO and Evans MR, 2004. *Campylobacter* infection in Gloucestershire: an ecological study. Health Protection Agency, Annual Conference. 13-15 September 2004, Warwick, UK.

Painter J, 2006. Estimating attribution of illnesses to food vehicle from reports of foodborne outbreak investigations. Society for Risk Analysis, 2006 Annual Meeting, 3-6 December 2006, Baltimore, MD.

Pebody RG, Ryan MJ and Wall PG, 1997. Outbreaks of *Campylobacter* infection: rare events for a common pathogen. Commun Dis Rep CDR Rev, 7, R33-7.

Petersen L, Nielsen EM, Engberg J, On SL and Dietz HH, 2001. Comparison of genotypes and serotypes of *Campylobacter jejuni* isolated from Danish wild mammals and birds and from broiler flocks and humans. Appl Environ Microbiol, 67, 3115-21.

Piddock LJ, 1995. Quinolone resistance and *Campylobacter* spp. J Antimicrob Chemother, 36, 891-8.

Pires SM, 2009. Attributing human salmonellosis and campylobacteriosis to animal, food and environmental sources. PhD thesis. ISBN 978-87-7611-311-7. University of Copenhagen, Faculty of Life Sciences, Copenhagen.

Pires SM, Evers EG, Pelt Wv, Ayers T, Scallan E, Angulo FJ, Havelaar A and Hald T, 2009. Attributing the human disease burden of foodborne infections to specific sources. Foodborne Pathogens and Disease, 6, 417-424.

Pires SM and Hald T, 2010. Assessing the Differences in Public Health Impact of *Salmonella* Subtypes Using a Bayesian Microbial Subtyping Approach for Source Attribution. Foodborne Pathogens and Disease, 7, In Press.

Pires SM, Nichols G, Whalstrom H, Kaesbohrer A, David J, Spitznagel H, Van Pelt W, Baumann A and Hald T, 2008. *Salmonella* Source Attribution in Different European Countries. Food Micro 2008. Aberdeen, Scotland, UK.

Pirie R, Cressey P and Lake R 2009. Annual report concerning foodborne disease in New Zealand 2006. Report of the Institute of Environmental Science & Research Limited, Christchurch, New Zealand. Client Report FW08015.

Powell L, Clifton-Hadley FA, Lawes J, Rodgers J and Vidal A, 2009. *Campylobacter* in broilers: Results from a UK National Prevalence Survey carried out in 2007. 15th International Workshop on *Campylobacter*, *Helycobacter* and related organisms. 2-5 September 2009, Niigata, Japan.

Pritchard JK, Stephens M and Donnelly P, 2000. Inference of population structure using multilocus genotype data. Genetics, 155, 945-59.

Ravel A, Greig J, Tinga C, Todd E, Campbell G, Cassidy M, Marshall B and Pollari F, 2009. Exploring Historical Canadian Foodborne Outbreak Data Sets for Human Illness Attribution. Journal of Food Protection, 72, 1963-1976.

Ribeiro CD and Frost JA, 2000. Family clusters of *Campylobacter* infection. Commun Dis Public Health, 3, 274-6.

Ribot EM, Fitzgerald C, Kubota K, Swaminathan B and Barrett TJ, 2001. Rapid pulsed-field gel electrophoresis protocol for subtyping of *Campylobacter jejuni*. J Clin Microbiol, 39, 1889-94.

Rosef O, Kapperud G, Lauwers S and Gondrosen B, 1985. Serotyping of *Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter laridis* from domestic and wild animals. Appl Environ Microbiol, 49, 1507-10.

Rosenquist H, Boysen L, Galliano C, Nordentoft S, Ethelberg S and Borck B, 2009. Danish strategies to control *Campylobacter* in broilers and broiler meat: facts and effects. Epidemiol Infect, 137, 1742-50.

Saenz Y, Zarazaga M, Lantero M, Gastanares MJ, Baquero F and Torres C, 2000. Antibiotic resistance in *Campylobacter* strains isolated from animals, foods, and humans in Spain in 1997-1998. Antimicrob Agents Chemother, 44, 267-71.

Sargeant JM, Rajic A, Read S and Ohlsson A, 2006. The process of systematic review and its application in agri-food public-health. Preventive Veterinary Medicine, 75, 141-151.

Schouls LM, Reulen S, Duim B, Wagenaar JA, Willems RJ, Dingle KE, Colles FM and Van Embden JD, 2003. Comparative genotyping of *Campylobacter jejuni* by amplified fragment length polymorphism, multilocus sequence typing, and short repeat sequencing: strain diversity, host range, and recombination. J Clin Microbiol, 41, 15-26.

Sheppard SK, Dallas JF, Strachan NJC, MacRae M, McCarthy ND, Wilson DJ, Gormley FJ, Falush D, Ogden ID, Maiden MCJ and Forbes KJ, 2009. *Campylobacter* Genotyping to Determine the Source of Human Infection. Clinical Infectious Diseases, 48, 1072–8.

Sheppard SK, McCarthy ND, Falush D and Maiden MC, 2008. Convergence of *Campylobacter* species: implications for bacterial evolution. Science, 320, 237-9.

Siemer BL, Harrington CS, Nielsen EM, Borck B, Nielsen NL, Engberg J and On SL, 2004. Genetic relatedness among *Campylobacter jejuni* serotyped isolates of diverse origin as determined by numerical analysis of amplified fragment length polymorphism (AFLP) profiles. J Appl Microbiol, 96, 795-802.

Simonsen J, Frisch M and Ethelberg S, 2008. Socioeconomic risk factors for bacterial gastrointestinal infections. Epidemiology, 19, 282-90.

Skirrow MB, 1977. *Campylobacter enteritis*: a "new" disease. Br Med J, 2, 9-11.

Skirrow MB and Blaser MJ, 2000. Clinical aspects of *Campylobacter* infection. In: *Campylobacter* 2nd edition. Nachamkin I and Blaser MJ (editors). ASM, Washington D.C., 69-88.

Smith JL and Bayles D, 2007. Postinfectious irritable bowel syndrome: a long-term consequence of bacterial gastroenteritis. J Food Prot, 70, 1762-9.

Smith JM, Smith NH, O'Rourke M and Spratt BG, 1993. How clonal are bacteria? Proc Natl Acad Sci USA, 90, 4384-8.

Smith KE, Besser JM, Hedberg CW, Leano FT, Bender JB, Wicklund JH, Johnson BP, Moore KA and Osterholm MT, 1999. Quinolone-resistant *Campylobacter jejuni* infections in Minnesota, 1992-1998. Investigation Team. N Engl J Med, 340, 1525-32.

Smith T and Taylor MS, 1919. Some morphological and biological characters of the spirilla (*Vibrio fetus* N. Sp) associated with disease of the fetal membranes in cattle. J.Exp.Med, 30, 294.

Stafford RJ, Schluter P, Kirk M, Wilson A, Unicomb L, Ashbolt R and Gregory J, 2007. A multi-centre prospective case-control study of *Campylobacter* infection in persons aged 5 years and older in Australia. Epidemiol Infect, 135, 978-88.

Stafford RJ, Schluter PJ, Wilson AJ, Kirk MD, Hall G and Unicomb L, 2008. Population-attributable risk estimates for risk factors associated with *Campylobacter* infection, australia. Emerg Infect Dis, 14, 895-901.

Stern NJ, Hiett KL, Alfredsson GA, Kristinsson KG, Reiersen J, Hardardottir H, Briem H, Gunnarsson E, Georgsson F, Lowman R, Berndtson E, Lammerding AM, Paoli GM and Musgrove MT, 2003. *Campylobacter* spp. in Icelandic poultry operations and human disease. Epidemiol Infect, 130, 23-32.

Strachan NJC, Gormley FJ, Rotariu O, Ogden ID, Miller G, Dunn GM, Sheppard SK, Dallas JF, Reid TMS, Howie H, Maiden MCJ and Forbes KJ, 2009. Attribution of *Campylobacter* Infections in Northeast Scotland to Specific Sources by Use of Multilocus Sequence Typing. The Journal of Infectious Diseases, 199, 1205-8.

Suerbaum S, Lohrengel M, Sonnevend A, Ruberg F and Kist M, 2001. Allelic diversity and recombination in *Campylobacter jejuni*. J Bacteriol, 183, 2553-9.

Swift L and Hunter PR, 2004. What do negative associations between potential risk factors and illness in analytical epidemiological studies of infectious disease really mean? Eur J Epidemiol, 19, 219-23.

Tam CC, Higgins CD, Neal KR, Rodrigues LC, Millership SE and O'Brien SJ, 2009. Chicken Consumption and Use of Acid-Suppressing Medications as Risk Factors for *Campylobacter* Enteritis, England. Emerging Infectious Diseases, 15, 1402-1408.

Threlfall EJ, Frost JA and Rowe B, 1999. Fluoroquinolone resistance in salmonellas and campylobacters from humans. British Medical Journal, 318, 943-944.

Thwaites RT and Frost JA, 1999. Drug resistance in *Campylobacter jejuni*, *C. coli*, and *C. lari* isolated from humans in north west England and Wales, 1997. J Clin Pathol, 52, 812-4.

Unicomb LE, Dalton CB, Gilbert GL, Becker NG and Patel MS, 2008. Age-specific risk factors for sporadic *Campylobacter* infection in regional Australia. Foodborne Pathog Dis, 5, 79-85.

Vally H, Hall G, Scallan E, Kirk MD and Angulo FJ, 2009. Higher rate of culture-confirmed *Campylobacter* infections in Australia than in the USA: is this due to differences in healthcare-seeking behaviour or stool culture frequency? Epidemiol Infect, 137, 1751-8.

van Hees BC, Veldman-Ariesen MJ, de Jongh BM, Tersmette M and van Pelt W, 2007. Regional and seasonal differences in incidence and antibiotic resistance of *Campylobacter* from a nationwide surveillance study in The Netherlands: an overview of 2000-2004. Clin Microbiol Infect, 13, 305-10.

Van Pelt W, Havelaar A, Westra P and Wagenaar JA, 2009. Strong regional reduction of camplylobacteriosis during and after avian influenza poultry farm culling. A model for future intervention studies at primary production? 15th International Workshop on *Campylobacter*, *Helycobacter* and related organisms. 2-5 September 2009, Niigata, Japan.

Van Pelt W, Van de Giessen AW, Mevius DJ, Vennema H, Koopmans M, Van Asten L and van Duynhoven YTHP, 2006. Trends in gastro-enteritis van 1996-2005. Toename van ziekenhuisopnames en sterfte: een toenemende rol.van virale infecties? Infectieziekten Bulletin, 17, 364-369.

van Vliet AHM and Ketley JM, 2001. Pathogenesis of enteric *Campylobacter* infection. Journal of Applied Microbiology, 90, 45S-56S.

Velazquez JB, Jimenez A, Chomon B and Villa TG, 1995. Incidence and transmission of antibiotic resistance in *Campylobacter jejuni* and *Campylobacter coli*. J Antimicrob Chemother, 35, 173-8.

Vellinga A and Van Loock F, 2002. The dioxin crisis as experiment to determine poultry-related *Campylobacter enteritis*. Emerg Infect Dis, 8, 19-22.

Wassenaar TM, 1997. Toxin production by *Campylobacter* spp. Clin Microbiol Rev, 10, 466-76.

Wassenaar TM and Newell DG, 2000. Genotyping of *Campylobacter* spp. Appl Environ Microbiol, 66, 1-9.

Wheeler JG, Sethi D, Cowden JM, Wall PG, Rodrigues LC, Tompkins DS, Hudson MJ and Roderick PJ, 1999. Study of infectious intestinal disease in England: rates in the community, presenting to general practice, and reported to national surveillance. The Infectious Intestinal Disease Study Executive. BMJ, 318, 1046-50.

Wieland B, Wittwer M, Regula G, Wassenaar TM, Burnens AP, Keller J and Stark KD, 2006. Phenon cluster analysis as a method to investigate epidemiological relatedness between sources of *Campylobacter jejuni*. J Appl Microbiol, 100, 316-24.

Williman J, Lim E, Pirie R, Cressey P and Lake R 2009. Annual report concerning foodborne disease in New Zealand 2008. Report of the Institute of Environmental Science & Research Limited, Christchurch, New Zealand. Client Report FW09062.

Wilson DJ, Gabriel E, Leatherbarrow AJ, Cheesbrough J, Gee S, Bolton E, Fox A, Fearnhead P, Hart CA and Diggle PJ, 2008. Tracing the source of campylobacteriosis. PLoS Genet, 4, e1000203.

Wingstrand A, Neimann J, Engberg J, Nielsen EM, Gerner-Smidt P, Wegener HC and Molbak K, 2006. Fresh chicken as main risk factor for campylobacteriosis, Denmark. Emerg Infect Dis, 12, 280-5.

APPENDICES

A. MICROBIAL SUBTYPING METHODS

This Appendix summarises the literature on subtyping *C. jejuni*, and the applications of this subtyping. A wide range of approaches to subtype *C. jejuni* have been developed and applied as outlined below. Identical subtypes have been used as evidence for a common recent origin in attempts to detect outbreaks, to define which cases belong to outbreaks detected by other means, and to assess whether different clonal groups dominate particular niches. Source prediction has been undertaken on both local and larger scales and has involved attempts to predict source of human and chicken infections. Although there has been a clear progression from phenotyping to non-sequence-based genotyping to sequence-based subtyping all methods continue to be used. Particular attention is given to a thorough treatment of multi-locus sequence typing (MLST).

There have been few comprehensive comparisons of available subtyping methods using selected sets of isolates but CAMPYNET[16] established an internationally available set of 100 *Campylobacter* strains from multiple sources and countries for the purposes of standardisation and harmonisation of methods. These strains have been extensively used and remain available through Med-Vet-Net[17].

1. Phenotyping

1.1. Laboratory methods of phenotyping

Serotyping, based on heat-labile antigens (HL) (Lior et al., 1982) or heat-stable antigens (HS) (Penner and Hennessy, 1980), and phagetyping (Grajewski et al., 1985; Frost et al., 1999; Patton et al., 1991) have been the main phenotypic methods applied separately or in combination. As well as being technically demanding, poor and variable typeability (the proportion of isolates for which a subtype can be determined) is inherent to the serotyping methods applied to *C. jejuni* (Wassenaar and Newell, 2000). Reproducibility has also been poor and although some modifications have reduced the contribution of laboratory limitations to this problem (Frost et al., 1998) serotype is itself relatively unstable and can change during the course of a single host infection (Hanninen et al., 1999). Serotype is not strongly linked to more global assessments of phylogenetic relationships (Hopkins et al., 2004) suggesting that it does not act as a reliable indicator of clonal relatedness in *C. jejuni*. Lack of discrimination, with just 10 serotypes including 53% of isolates in one large study (Frost et al., 1998) is a further major limitation for discriminatory purposes. This may, in part, be due to the geographical and temporal restriction of the sample used to define the origin panel of isolates for the most widely applied Penner scheme (Penner and Hennessy, 1980), which limits the diversity indexed among these reference serotypes. Increasing the number of phages used in phagetyping schemes has improved the typeability and discrimination offered by this approach (Frost et al., 1999; Patton et al., 1991). The combination of phagetyping with serotyping improves discrimination and became the UK reference laboratory approach (Frost et al., 1999).

1.2. Applications of phenotyping

Phenotyping has been widely applied and has produced some substantive contributions. Several serotypes (including HS 19 and HS 41) have been identified which confer an increased risk of Guillain-Barré syndrome (GBS) subsequent to infection (Lastovica et al., 1997; Mishu and Blaser, 1993). The distribution of serotypes has been shown to vary with the age and geographic location of patients (Schonberg-Norio et al., 2006). However, this analysis did not differentiate between fixed

16 See http://campynet.vetinst.dk
17 See http://www.medvetnet.org

geographical differences or heterogeneity due to links between clustered cases and used statistical approaches that did not account for such plausible sources of variance, making it difficult to interpret the origin of the effect as being due to clustering or another form of population structuring.

Several studies have assessed *Campylobacter* ecology in terms of host association, in some cases including human isolates. A comparison of serotypes from wildlife isolates (hedgehogs, squirrels, other mammals and birds, n=47), broiler chickens (n=29) and humans (n=44) showed lesser differences between human and broiler isolates than between either of these species and the wildlife strain collection. Some serotypes were present in all three groups and further differentiation by genotypic analyses supported these isolates as being clonally related (Petersen et al., 2001). An early assessment of serotype among both *C. jejuni* and *C. coli* in Norway, using a different scheme, also suggested that human isolates were more closely related to those from poultry than those from wild birds, flies or pigs, but with relatively minor differences (Rosef et al., 1985). Studies in New Zealand have shown similar serotype distributions in humans at different times and identified the same serotypes frequently in farm animals, particularly ruminants, but less commonly in wild ducks (Devane et al., 2005; Garrett et al., 2007). A UK study on serotypes in humans noted an increased rate of reporting drinking bird-pecked milk in individuals infected with strains of HS6, but this was the only positive association from many hypotheses tested and test statistics did not consider multiple comparisons (Sopwith et al., 2003). Use of serotyping to determine the origin of infections in a large study in chickens (3,304 isolates) was limited by lack of variation with three serotype complexes accounting for 58% of isolates and ten for 85% (Pearson et al., 1996).

1.3. Summary of phenotyping and its application

Phenotyping has thus far provided evidence that at least one sequela of infection is associated with subtype, that there is partial differentiation between *C. jejuni* in different host species, that human subtypes resemble those strains isolated from chicken and other farm animals more than they do from wild animals, and that there may be overlapping populations between different host groups. No phenotyping study has reported a quantitative attribution to source but the reported overlap in apparently clonally-related isolates from different species would prove a complication to attribution approaches. In part due to lack of comparability, with different or modified schemes being used as well as difficulties in between-laboratory standardisation, no joint analysis has been made of different studies. The limited capacity for joint analysis is a barrier to this approach making a more substantial contribution.

2. Genotyping other than sequence subtyping

A wide range of methods assaying variation across the genome involve the generation of DNA fragments using restriction enzymes (e.g. Pulsed-Field Gel Electrophoresis (PFGE) (Wassenaar and Newell, 2000; Yan et al., 1991)) or amplification of DNA (e.g. Randomly Amplified Polymorphic DNA (RAPD) (Wassenaar and Newell, 2000)) or a combination of both such as Amplified Fragment Length Polymorphism (AFLP) (Duim et al., 1999). These approaches generate band patterns representing the genome wide distribution of the binding sites of the enzymes or primers used. Some methods focus on a more restricted part of the genome. Several distinct approaches to polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) subtyping of the *flaA* gene (Wassenaar and Newell, 2000) or other genes (Ragimbeau et al., 1998) have been developed giving much more detailed information from this short but highly variable region of DNA. More recently, with the generation of whole *Campylobacter* genome sequences, microarray methods have been developed based on hybridisation with short gene fragments (Champion et al., 2005).

Standardisation and reproducibility of such techniques, though difficult, have been partly achieved first through CAMPYNET and continued through Med-Vet-Net and, in particular for PFGE, through PulseNet (Gerner-Smidt et al., 2006; Ribot et al., 2001).

2.1. PFGE

PFGE subtype can be unstable and can change substantially due to genome rearrangement during a single infection (Hanninen et al., 1999; Scott et al., 2007) and during certain environmental stresses (Ridley et al., 2008). This instability is strain dependant. Thus for some, but not all, strains, *Campylobacter* phylogenies inferred from PFGE data may be unreliable (with, for example, isolates from different species clustering together (Keller et al., 2007)). Nevertheless, it is by far the most widely used of these approaches, it is considered the gold standard for discriminatory subtyping (Gerner-Smidt et al., 2006; Sails et al., 2003b) and is particularly useful for epidemiological studies over short temporal and geographical distances.

2.2. Applications of PFGE

2.2.1. Outbreak investigation

In two outbreaks, which had been identified by other means, PFGE was reported as useful in identifying whether cases belonged to the outbreak (Fitzgerald et al., 2001; Kuusi et al., 2005). Three studies performed to evaluate PFGE have confirmed this capacity to confirm cases as belonging to an outbreak by comparing a range of sporadic isolates and isolates from several outbreaks (Mellmann et al., 2004; Sails et al., 2003b; Suzuki et al., 1994). The most extensive use of PFGE to support outbreak investigation has been within the USA PulseNet, where more than 2,500 profiles have been identified using standardised methods (Gerner-Smidt et al., 2006). A consensus view from this experience is that it is a useful approach to support the investigation of detected outbreaks but not useful as a means to detect *C. jejuni* outbreaks (Gerner-Smidt et al., 2006; Hedberg et al., 2001). This is in contrast to the PulseNet findings for other bacteria where PFGE has been used to detect outbreaks (Gerner-Smidt et al., 2006). However, it largely reflects the presence of multiple *Campylobacter* strains within outbreaks rather than a problem with the technique. PulseNet collaborators only used the *SmaI* restriction enzyme but a proportion of strains *SmaI* refactory. In these cases, CAMPYNET recommend that *KpnI* also be used. A combination of the two enzyme profiles is more discriminatory (Michaud et al., 2001; Garrett et al., 2007) than either enzyme in isolation.

Studies from New Zealand (Garrett et al., 2007) and Canada (Michaud et al., 2005) each investigated 183 cases by PFGE. The New Zealand study was entirely retrospective using available data and identified clusters of subtypes. Some of these clusters also showed temporal clustering (although not formally analysed) and some genotypes may also have clustered by area of residence and reported occupation. However, further investigation was not done to assess if these cases had true epidemiological links. Nevertheless, the conclusion was that similar PFGE patterns may identify epidemiologically meaningful outbreaks arising from a common source. The Canadian study included both retrospective and prospective components. Outbreaks identified by other means were confirmed as being of identical subtypes. However clusters identified by subtyping did not yield evidence for a common source on further investigation. Since most clusters were small (2 to 3 cases) identification of source was difficult and conclusion was that following such small genotypic clusters is unjustified (Michaud et al., 2005), which is consistent with our perceptions of outbreak data.

2.2.2. Source attribution

Groups of PFGE subtypes in human cases appear to vary over time and according to patient characteristics (Hook et al., 2004), which might indicate evidence for different sources. Host association of subtypes has been examined in several studies. In Sweden isolates from wild birds, broilers and humans were analysed jointly by a UPGMA cluster analysis. Two main clusters were produced. Sixty of 73 gull isolates were in one genotypic cluster, mostly in an almost exclusively gull-origin sub-cluster, while human and chicken isolates were more widely scattered and generally closer

to each other than to gull isolates (Broman et al., 2002). A comparison of 49 human isolates with 87 from a range of wild birds identified only two isolates in wild birds that were similar to those from humans, one each from a starling and a blackbird (Broman et al., 2004). A Finnish study of isolates from chickens and humans in the seasonal peaks during 1996-1998 showed a wider range of subtypes in chickens that mainly persisted across the three years. Fewer subtypes were isolated from humans with more variation from year to year. However most human isolates represented subtypes also identified in chickens (Hanninen et al., 2000). A Danish study using phenotyping and PFGE on a wide range of wild animal, as well as human and broiler, isolates identified fourteen clusters with similar PFGE patterns. Seven of these clusters included human isolates (Petersen et al., 2001). Four of the subtypes with human isolates also included broiler isolates. The only wild animal or bird isolates (hedgehog, fox, deer, squirrel, duck, buzzard, gull, sparrow hawk, hare, seal, pheasant, rook, raven, magpie or seagull) to share a subtype with human isolates was from a hedgehog. There was some sharing of subtypes between broiler and wild animals and between different wild animal species with four wild animal species (fox, squirrel, duck and buzzard) identified as carrying the most widely distributed subtype. These results are consistent with the phenotyping of this sample.

A New Zealand comparison of isolates from humans, water, chicken and veterinary origins sampled during winter (August) and summer (February) found clear differences in subtypes between the two time periods. Human isolates mostly shared subtypes with either chicken or water subtypes. No attribution analysis was performed and the tabulated data suggests that little accurate attribution would be possible. A further study in Curacao combined case control epidemiology with PFGE of isolates. Generally there was no association between subtype and risk factors with the possible exception of individuals owning a deep domestic well being more likely to have infection with one PFGE pattern (Endtz et al., 2003). A large Canadian broiler study (involving 93 flocks of chickens on 57 farms) showed a marked diversity of PFGE patterns, including some diversity of subtypes on individual farms, but with some identical subtypes at different periods and on different farms. Approximately 20% of human genotypes were identical to clones identified among poultry (Nadeau et al., 2002). No other host samples were available.

2.2.3. Summary of PFGE and its application

Like phenotyping, PFGE indicates that there is partial differentiation of *C. jejuni* populations by host species, and that human isolates are closer to those from broilers than of wild animal origin. Also like phenotyping, combined analysis of the many PFGE studies described above is not possible given lack of standardisation. Although outbreak identification has not been well supported by PFGE, the method appears useful in including or excluding cases from outbreaks already under investigation. This poor performance in outbreak detection suggests either that a very highly discriminatory methods may be needed to support outbreak identification for this pathogen or that outbreaks may really be very rare events.

2.3. AFLP

Five AFLP studies have assessed host association and possible sources of human infection. A Danish study considering possible sources of human infection reported that most clones infecting humans were also isolated from several non-human animal sources making it difficult to infer source based on subtype (Siemer et al., 2004). A UK study found relatively marked differences between pig and poultry *C. coli* strains but no clear clonal separation of *C. jejuni* in samples from poultry, cattle, swine and humans, although relative prevalences differed among some AFLP defined clones (Hopkins et al., 2004). A Swiss study identified quite marked separation between poultry, pets, cattle and humans and there was some evidence for cattle-associated clones identified on multiple farms. Most (47 of 64) human isolates in this study were in human-only clones. In a Dutch study, including only human and chicken isolates, 3 main clusters were identified, each of which included isolates of both human and chicken origin with no cluster restricted to either host (Duim et al., 1999). In a second Dutch study,

including human, chicken and cattle isolates, again no convincing evidence was found for a host-association using AFLP (Schouls et al., 2003). The information on subtype provided by AFLP has not therefore been shown to be useful in predicting sources of human infection. Nevertheless, because of the high discriminatory power of the technique and its insensitivity to genetic instability, AFLP has been used in the identification of clonal stability over time (Manning et al., 2001), evaluation of environmental contamination from broiler flocks on farm (Johnsen et al., 2006a) and at slaughter (Johnsen et al., 2006b, Johnsen et al., 2007), as well as evaluating genotype-specific survival following environmental stress (Wieland et al., 2006).

2.4. Single gene RFLP

FlaA subtyping was the earliest genotyping scheme developed. At least seven *flaA* restriction fragment length polymorphism (RFLP) schemes have been described (Wassenaar and Newell, 2000). Several of these have been compared for discriminatory power and reproducibility in CAMPYNET studies (Harrington et al., 2003). Genetic events can substantially alter *flaA* genes (Harrington et al., 1997; Mellmann et al., 2004) and, as a consequence, these genes have minimal relationship to the clonal history of an isolate and even show evidence for substantial cross-species recombination (Dingle et al., 2005). As a result, although allowing a rough prediction of whether or not isolates are identical, *flaA* RFLP methods are considered not useful for human epidemiological studies. Nevertheless, such events are observed infrequently in the field and over short periods of time, and *fla* subtyping, which is cheap and rapid, has proved very valuable in on-farm epidemiological studies for example screening large numbers of isolates to identify potential sources of poultry flock infection.

2.5. Microarrays

More recently microarray approaches have allowed the capture of more detailed genome wide information, such as gene content, by assaying whether an isolate has very similar genes to those from a fully sequenced isolate on a gene by gene basis (Champion et al., 2005). This approach uses hybridisation with a cut-off point determining whether the isolate under study is classified as having a gene that is sufficiently similar to the probe on the microarray. The value of early arrays was limited by the genome data available. With each *Campylobacter* genome sequenced, the variability of the pan-genetic complement is substantially increasing and new microarrays are being designed to incorporate this information. Integrating data produced from different generations of arrays will provide a further challenge. In common with the genome wide approaches that produce band patterns, there is a lack of good models describing the genetic processes producing the observed patterns of results, in this case gene gain and loss. This limits the quality of inference available from microarray data (Gressmann et al., 2005). One study reported that this approach identified separate livestock and non-livestock clades with most human cases fitting into the livestock clade (Champion et al., 2005). However, this study lacked an adequate sample of non-human isolates, used an array based on a single genome study, and did not take account of the population structure already identified by sequence-based subtyping methods (Dingle et al., 2001a; Dingle et al., 2002). The importance of the use of unbiased populations has been shown for other bacterial species (Gressmann et al., 2005). To date microarray analysis has, therefore, not contributed usefully to the understanding of the *C. jejuni* population structure or its epidemiology. Once arrays adequately representing the *C. jejuni* pan-genome become available and are consistently applied, this technology will allow assessment of gene content, although large scale sequencing approaches (e.g. Solexa, 454 etc) may have superseded this approach by that time.

2.6. Summary of non-sequence genotyping and its application

Among non-sequence based genotyping, PFGE has been the most widely used and most useful approach, and has supported case definition in outbreak studies as well as giving relatively consistent results in studies of source attribution suggesting a large role for farm animals, and poultry in

particular, in the origin of human infections. The applicability of all these methods to quantitative source attribution has been restricted by lack of comparability between studies which prevents efficient combined analysis of different studies. The methods have had variable success for outbreak detection or the provision of insight leading to the understanding of bacterial population structure and biology.

3. Sequence subtyping

3.1. Sequencing single highly variable gene fragments

Sequencing of a variable portion (the short variable region (SVR)) of the *flaA* gene is the most common single gene subtyping approach to date (Dingle et al., 2005; Harrington et al., 1997; Meinersmann et al., 1997), although other genes have also been used (Zhang et al., 2000). Two factors limit the inference that can be drawn from *flaA* sequencing. Firstly, identical alleles of the *flaA* gene fragment have been found in *C. jejuni* and the related species *C. coli* (Dingle et al., 2005). Secondly, substantial change can occur in a single recombination so that closely related isolates can have very different *flaA* sequences (Harrington et al., 1997). Sequence-based approaches have been useful in explicitly demonstrating such limitations of the use of any single gene in a frequently recombining species. However, the implication is that sequencing of the *flaA* gene, without a wider genotypic context, has no substantial advantage over non-sequence subtyping of this gene. In other bacterial species, systems using sequences from two or more variable genes (Martin et al., 2004), or from one or more variable genes in the context provided by a more stable subtyping approach, such as MLST (Feavers et al., 1999) have been applied and are more reliable in both discriminating between closely related isolates and avoiding the false identification of apparently close relatedness between distant isolates sharing a common sequence at a single locus.

3.2. Whole genome sequencing

The first full genome sequence of a *C. jejuni* strain was reported in 2000 (Parkhill et al., 2000). Since then other full sequences have become available (Duong and Konkel, 2009; Fouts et al., 2005). Currently over 20 *Campylobacter* spp. genome sequences are available or at near completion. The increasing availability of high-throughput sequencing approaches means that whole genome sequences may soon be feasible even for large sample collections (Meyer et al., 2008). This will present large opportunities and analytical challenges which remain speculative at present.

3.3. MLST of *C. jejuni*

MLST of bacteria involves sequencing the forward and reverse strands of several, typically seven, gene fragments (Dingle et al., 2001a; Maiden et al., 1998; Maiden, 2006). Gene fragments are chosen to allow (1) subtyping of all, or virtually all, members of the target group of bacteria and (2) identification of genetic relatedness between isolates using analysis based on genetic processes involved in evolution. The genes chosen encode for essential metabolic functions. This means that they are likely to be present in all isolates, assuring the aim of typeability. A consequence of this is that the genes are under stabilising selection, which limits the diversity available from each gene fragment and therefore lowers the efficiency of identification of differences between isolates. Based on other bacterial species, the use of seven genes is assumed to provide sufficient information to allow isolates to be grouped according to a moderately detailed genealogy. Closely related isolates, sharing identical alleles at several of the loci, are thus identified as belonging to a particular clonal complex (CC) or even having an identical sequence subtype (ST) across all 7 loci. Isolates identified as belonging to the same CC are very likely to share a more recent common ancestor with each other than with isolates which are not members of the clonal complex (Maiden et al., 1998; Smith et al., 1993).

Analysis of MLST data can take advantage of information from genes at multiple separate loci on the genome. This enables the identification of overall population structure, the place of an isolate in that structure, and estimates the contribution of different effects such as mutation, recombination (swapping of segments of DNA between lineages of bacteria), selection and neutral processes, such as genetic drift, to the generation of genetic diversity, maintenance of species characteristics and the shaping of population structure (Didelot and Falush, 2007; Falush et al., 2003). This capacity for analysis, using models based on the understanding of basic genetic processes contrasts with subtyping schemes using empirical subtyping data where no clear model of the processes producing change is available.

Three MLST schemes for *C. jejuni* have been published (Dingle et al., 2001a; Manning et al., 2003; Suerbaum et al., 2001). One of these has become widely applied (Dingle et al., 2001a). Although the others have not been used in subsequently published work to date, this does not mean that these are not appropriate for widespread use, just that one scheme has self-generated dominance. The large scale and increasing application of a single scheme (Meinersmann et al., 2003; Manning et al., 2003; Wareing et al., 2003; Dingle et al., 2002; Colles et al., 2003; Sails et al., 2003a; Sails et al., 2003b; Schouls et al., 2003; Mellmann et al., 2004; Dingle et al., 2005; Best et al., 2004; French et al., 2005; Clark et al., 2005; Fitch et al., 2005; Miller et al., 2005; Miller et al., 2006; Karenlampi et al., 2007; Kinana et al., 2006; Djordjevic et al., 2007; D'Lima C et al., 2007; Mickan et al., 2007; Sopwith et al., 2006; Kinana et al., 2007; O'Reilly et al., 2006; Allen et al., 2007; McCarthy et al., 2007; Litrup et al., 2007; Duim et al., 2003; Hepworth et al., 2007; Clark et al., 2007; Parker et al., 2007; Price et al., 2006; Feil et al., 2004; Thakur et al., 2006) since first described in 2001 (Dingle et al., 2001a) allows direct comparison between, and combined analysis of data from, different studies using a standardised approach. This is further supported by an accessible public database (Jolley et al., 2004). The position on the genome of the seven gene fragments used in this scheme, which has also been applied to *C. coli* (Dingle et al., 2005), are shown in Figure 1. The size and the detailed position of each gene fragment on the *Campylobacter* genome is indicated in Table 1. It is notable that at least in this genome sequence the selected genes tend to be clustered in part of the genome and this might be a disadvantage.

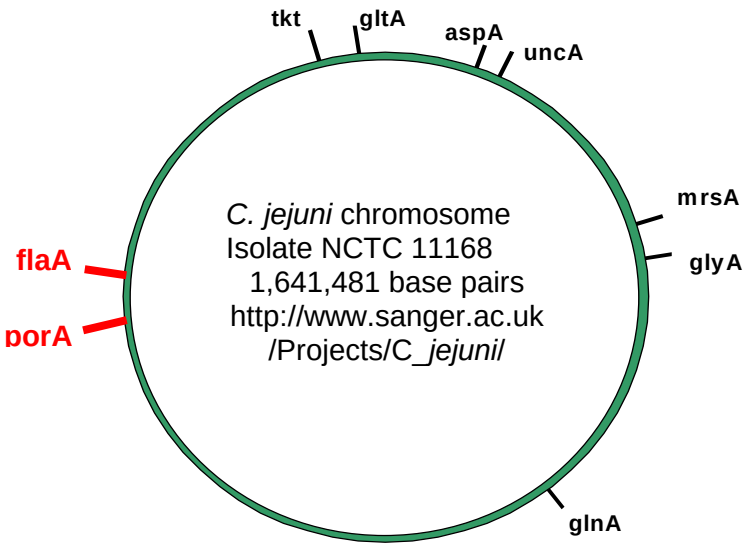

Figure 1: Position of MLST loci (Dingle et al., 2001a) (in black) and selected antigen genes (Dingle et al., 2008) (in red) on the NCTC11168 genome.

Table 1: Location and size of MLST (Dingle et al., 2001a) loci on NCTC11168 *C. jejuni* genome

MLST gene	Identifier and position on NCTC11168 genome		Base pairs
aspA	Cj0087	96692 to 97168	477
*uncA**	Cj0105	112163 to 112651	489
mrsA / pgm†	Cj0360	327773 to 328270	498
glyA	Cj0402	367573 to 368079	507
glnA	Cj0699c	657609 to 658085**	477
tkt	Cj1645	1569415 to 1569873	459
gltA	Cj1682c	1604529 to 1604930**	402

* *uncA* renamed to *atpA* and now identified as such on the NCTC1118 genome

† *"pgm"* most similar to *E. coli mrsA.* Currently not named on the NCTC1118 genome having been previously identified as both *pgm* and *mrsA*

** *glnA* and *gltA* are coded on the negative sense DNA strand

Several papers have described single nucleotide polymorphism (Best et al., 2004; Best et al., 2005; Price et al., 2006) or other (Djordjevic et al., 2007; O'Reilly et al., 2006) methods to shortcut sequencing requirements to obtain groupings similar to those defined by MLST. These have generally produced a rapid approach capable of moderate accuracy. However, they are not capable of indexing further diversity, which is inherent to the standard application of MLST through new allele discovery and the identification of new allele combinations. There are no published reports of the practical application for scientific or public health purposes of these short cut approaches and they are not considered further here.

The reported applications of MLST to the population biology, ecology, and epidemiology of *C. jejuni* are reviewed below. MLST refers to the most widely applied scheme (Dingle et al., 2001a) unless otherwise specified.

3.4. Application of MLST to the population genetics and biology of *C. jejuni*

3.4.1. Population genetic parameters

Analysis using MLST has shown that *C. jejuni* is weakly clonal. This was reported using index of association analyses in the first report of the most widely used MLST scheme (Dingle et al., 2001a), with largely consistent results from analyses in the other two schemes that have been developed (Manning et al., 2003; Suerbaum et al., 2001). Initial estimates for the basic population genetic parameters suggested that recombination events were eight-fold more common than mutations with an average fragment length estimated at 3.3 kb (Schouls et al., 2003). Later model-based population genetic analysis of data, from a structured sample of a local area and it's animals (French et al., 2005), estimated the recombination rate as being similar to the mutation rate in *C. jejuni* and the mean recombination fragment size as in the order of 500-750 bp long and similar in each host species studied (Fearnhead et al., 2005). Although conclusions about the lack of evidence for large recombination fragments in this study used an erroneous gene order, this is unlikely to have made a major contribution to these estimates so that current combined evidence supports mainly relatively short recombination fragments, on average in the order of hundreds of base pairs.

3.4.2. Population structure and diversity within *C. jejuni*

A comparison of MLST and MultiLocus Enzyme Electrophoresis (MLEE) among a diverse set of *C. jejuni* isolates confirmed the similarity of grouping obtained by each method (Sails et al., 2003a) as expected from the design of MLST (Dingle et al., 2001a; Maiden et al., 1998). The large collections of isolates, which have been MLST typed, have demonstrated substantial diversity with 4218 unique STs identified in the combined *C. jejuni* / *C. coli* database (at 25 November 2009) and 43 defined

clonal complexes. The most diverse clonal complexes within *C. jejuni* are ST-21 complex including 409 STs and ST-45 complex with 229 STs (at 13 November 2009). MLST has also been used to describe the phylogenetic relationship of the subspecies *C. jejuni* subsp. *doylei* showing that a geographically diverse set of *C. jejuni* subsp. *doylei* isolates formed a phylogenetically distinct cluster within *C. jejuni* (Parker et al., 2007) consistent with *C. jejuni* subsp. *doylei* isolates sharing a more recent common ancestor with each other than with other *C. jejuni* isolates.

3.4.3. Gene flow between *Campylobacter* species

The relationship between *C. jejuni* and *C. coli* has also been analysed using MLST (Dingle et al., 2005). This has shown the presence of hybrid forms, that these result from recombination between *C. jejuni* and *C. coli* (Dingle et al., 2005; Meinersmann et al., 2003; Schouls et al., 2003), that such recombination may be particularly frequent among isolates obtained from ruminants (Meinersmann et al., 2003), and that it can be found in isolates on different continents (Kinana et al., 2007). Recombination with other species appears to be less common (Meinersmann et al., 2003) but does occur. There is evidence for lateral gene transfer between *C. jejuni* and *C. lari* (Miller et al., 2005) and unidentified cross species imports have been reported among Danish *C. coli*, as well as imports from *C. jejuni* (Litrup et al., 2007).

3.5. MLST and the distribution of genotypic and phenotypic characteristics

Population structure indicated by MLST has been used, along with other genotype information, to describe the distribution of *porA* gene variants (Clark et al., 2007), to assess the stability of the flagellar genes *flaA* and *flaB* (Mellmann et al., 2004), and to identify that gene content differences mainly map to the clonal population structure rather than to the host of origin (Hepworth et al., 2007). Isolates from patients with Guillain-Barré Syndrome are genetically diverse and are found in several MLST-defined clonal complexes (Dingle et al., 2001b), consistent with earlier findings using MLEE (Engberg et al., 2001). Most studies considering antibiotic resistance in the light of MLST-defined population structure have focussed on *C. coli* where they have shown patterns such as a clonal distribution of erythromycin resistance due to a 23S rRNA mutation (Chan et al., 2007) and susceptibility to other antibiotics (D'Lima C et al., 2007) among isolates from turkeys in the USA. In contrast some studies found a lack of strong clonal patterning of resistance to a range of antibiotics among *C. coli* from swine in the USA, even when the resistance had the same genetic basis (Thakur and Gebreyes, 2005), or of quinolone resistance in isolates from chicken in Senegal (Kinana et al., 2007). The associations in these papers were observational rather than based on formal analysis of resistance in relation to population structure.

3.6. MLST applied to the ecology of *C. jejuni* subtypes

The seven published studies describing the distribution of isolates by clonal complex across multiple hosts and one restricted to chicken isolates are summarised in Table 2. The table only includes the most commonly identified clonal complexes. ST-21 complex and ST-45 complex appear to be relatively widely distributed across many host species as well as abundant. Ruminants and chickens are the most usual sources of such strains, with ruminants slightly more dominant in ST-21 complex and chickens in ST-45 complex. However, these farm animals are the most sampled sources and isolation has also been reported from wild birds, other animals and environmental samples for both clonal complexes. ST-206 complex isolates have been reported frequently from both chicken and ruminant sources but not from wild animals or environmental samples. The importance of ruminant hosts for ST-61 complex, ST-42 complex and to an extent ST-48 complex is well supported across studies in different European countries, while ST-257 and ST-354 complexes are somewhat more common in isolates of chicken origin. ST-177 and ST-179 have been mostly reported in isolates from sand samples, although three ST-177 isolates from starlings and one from a pet have also been identified. One study restricted to chicken isolates is included as it is substantially separated

geographically from other isolates (Kinana et al., 2006). These Senegalese chicken isolates mostly fit into the chicken associated complexes (257 and 354) or the multi-host ST-21 complex, with one isolate in ST-42 complex which is usually more ruminant associated. At a quite different geographical level, a study within a 10 km square grid showed that isolates that were sampled near to each other were more likely to be identical than distant samples. Inference on whether this only operated at less than 1km or extended longer was dependent on the analytical model chosen limiting the precision of the inference that can be drawn (French et al., 2005).

A formal approach to attribution of *C. jejuni* to host of origin between chickens and ruminants (McCarthy et al., 2007) using the Structure algorithm (Falush et al., 2003) has also shown that genotype is more strongly associated with animal host species than with geography, even at an international level. This study also demonstrated that the signal of association with host is not entirely a clonal one. Genes acquired by recombination also predict host of association (McCarthy et al., 2007). This finding implies that "summary genotypes" cannot effectively predict origin and explains the basis for past difficulties in attributing human infection by *C. jejuni* to source using subtype (Evers et al., 2007; Neimann et al., 2003; Schouls et al., 2003). This population genetic approach to host attribution, of using individual gene information rather than summary subtype, has subsequently been applied using both the Structure algorithm (Sheppard et al., 2009) and an asymmetric island model (Sheppard et al., 2009; Wilson et al., 2008). Estimates of the origin of human *C. jejuni* infection using these approaches suggest that of the order of 60% to 80% of human infection is from chickens. However, these estimates rely on the assumptions that the reference populations used (to represent the possible host species of origin for human infection) were complete and representative, which are not true. The extent to which this affects the accuracy of the estimates is uncertain. Validating predictions of source of human infection using population genetic attribution models for multilocus sequence data against external information, such as exposure histories from cases, is an important outstanding area of work. Published work is therefore consistent with some geographical structuring of *C. jejuni* populations at a local level, but suggests that host association may be a much stronger effect than geography, even over large distances, and that this genetic imprint of host association may be useful in identifying the source of human infection at a population level. The direct comparability of MLST results between studies is central to enabling these inferences that rely on data from several independent projects.

Table 2: Host species of isolation of major clonal complexes of *C. jejuni* in published reports, 2001-2007.

Paper	Country	Size	Year	21	42	45	48	61	177/179	257	206	354	403	Other
Colles et al. (2003)	UK,	n=112	1990s	Rm St Ch	Rm	Ch Tu Rm St	Rm	Rm	St	Tu Rm St	Rm			
Dingle et al. (2002)	UK, NL mainly	n=313	Up to 2001	Rm Ch Sa	Rm	**Ch** Rm Sa	Rm Sa Ch	**Rm** Sa	Sa	Ch	Ch Rm	Ch	Rm	
Dingle et al. (2001a)	UK, NL	n=115	1990s	Rm Ch Sa		**Ch** Sa			Sa					
French et al. (2005)	UK	n=172	2000	**Rm** Bi Wa Ra	**Rm** Ra Wa Bi	**Rm Bi Wa** Ra So Ba		**Rm** Ra Wa						
Karenlampi et al. (2007)	Finland	n= 53	2003	Rm Ch		Ch Rm	Rm	Rm						ST-677 Ch
Kinana et al. (2006)	Senegal	n= 46	2000-2	Ch	Ch					Ch		Ch		
Manning et al. (2003)	UK mainly	n=215		**Rm Ch** Sw Pe	Rm	**Ch** Rm Pe	Rm Pe Ch	**Rm** Sw Gi	Pe	Ch Rm	**Rm** Ch	Rm	**Sw** Rm	ST-573 Ch
Schouls et al. (2003)	NL mainly	n=90	1990s	Rm Ch	Rm	Rm Ch	Rm Ch	Rm		Rm Ch				ST-46 Ch Rm

Ba Badger; Bi Wild bird – unspecified; Ch Chicken; Gi Giraffe; Pe Pet animal; Ra Rabbit; Rm Ruminant; Sa Sand; So soil; St Starling; Sw Swine; Tu Turkey; Wa Water.
Dominant hosts identified in bold type. Order of listing host gives a further estimate of most frequent source of isolation. Given variable sample numbers from each source in each study these are very approximate estimates of apparent host association.

3.7. MLST and the dynamics of infection in agricultural animals and settings

MLST has been used to identify the population dynamics of infection among successive chicken flocks in a study assessing the effect of phage susceptibility on broiler infection showing that phage resistance was mediated by the incursion of phage resistant subtypes rather than the *de novo* development of phage resistance (Connerton et al., 2004). A study describing the diversity and dynamics of ten housed broiler flocks in the UK used MLST to demonstrate the limited number of infecting subtypes at any one time, successive infection by different subtypes over time, contamination of water and the environment with similar subtypes and, on occasion, isolation of the subtypes infecting broilers from the breeder flocks or environment prior to the detection of infection in the broiler flock (Bull et al., 2006). The dynamics of chicken meat contamination during chicken processing used MLST to compare infecting subtypes and identified evidence for differences in subtypes isolated from caecal culture and carcass culture as well as for carry over of infecting subtype onto successive flocks at the same processing plant (Allen et al., 2007). Isolation from header tank water of *C. jejuni* that, in some cases, matched the contaminating subtype among chickens might have been interpreted as a possible route of entry of infection (Ogden et al., 2007), although both the lack of discrimination afforded by MLST alone and the lack of temporal studies to indicate a clear direction of causality limits lead the authors to question such a conclusion. All of these observations, and more, had been previously made using alternative molecular epidemiological approaches such as PFGE and *fla* typing. The use of MLST as an approach to trace infection in an agricultural setting is restricted, not least by lack of discriminatory power, so that the development of more reliable fine subtyping to complement the MLST subtype may be useful to support studies exploring the dynamics of *C. jejuni* infection on farms and during processing while also allowing comparison to, and joint analysis with, external datasets. In the meantime use of methods such as PFGE, AFLP etc., is more appropriate for such studies.

3.8. MLST applied to epidemiology

The main epidemiological functions for which MLST has been evaluated or applied are determination of host association as described above, outbreak investigation, comparison of trends in infecting subtypes over time or between populations, and integration in a case control study to guide subgroup analysis.

3.8.1. Outbreak investigation

Isolates that were part of a Canadian waterborne outbreak were identical. However, this subtype was also identified in non-outbreak isolates showing that is did not effectively discriminate outbreak isolates from unrelated isolates (Clark et al., 2005). Validation studies, each including several outbreaks, have also been completed (Mellmann et al., 2004; Sails et al., 2003b) giving similar results, with MLST inadequately discriminating to allow it's application in identifying which cases are outbreak related and which are not. A combination of MLST and highly variable gene subtyping should increase discrimination to allow this function, as it would provide discrimination at least equivalent to PFGE (Mellmann et al., 2004; Sails et al., 2003b). It is less certain whether an MLST plus "antigen gene fragments scheme" will be effective in outbreak detection, an important potential task for subtyping schemes in relation to *C. jejuni* where outbreak detection is uncommon. This approach of combining MLST with variable gene sequences is being developed (Dingle et al., 2008).

3.8.2. Trends between populations and over time

Collections of human isolates typed by MLST have been published from the UK (Dingle et al., 2002; Sopwith et al., 2006), the Netherlands (Dingle et al., 2002), Curacao (Duim et al., 2003), the USA (Fitch et al., 2005), Finland (Karenlampi et al., 2007), Australia (Mickan et al., 2007), and New

Zealand (McTavish et al., 2007) allowing direct comparison. The first large scale study of human isolates (n=501) was of an international collection but with many isolates from the UK (n=370) and the Netherlands (n=79), the large majority of which were isolated between 1990 and 1999, including 213 in 1991. This study showed that human infections included diverse isolates from 15 identified clonal complexes with ST-21, ST-45, ST-61, ST-48, and ST-257 complexes the most common; in that order (Dingle et al., 2002). A study of 234 isolates (Duim et al., 2003), which had been collected during a 1999/2000 case control study (Endtz et al., 2003) in Curacao, found many isolates in the same clonal complexes identified in Europe (Dingle et al., 2002). Seven of the 10 most frequent complexes from Curacao had already been identified, but the remaining 3 comprised novel ST-41, 508 and 657 complex isolates. Theses 3 clonal complexes comprised 61 isolates (26% of the total). Additionally, 41 isolates belonged to ST-403 complex, which is relatively rare in humans in Europe (Dingle et al., 2002). These ST-403 complex isolates were restricted to the November-February period of the Curacao study, consistent with either a seasonal or transient source.

An Australian case control study conducted between 1999 and 2001 provided 153 isolates, which were subsequently typed using MLST (Mickan et al., 2007). Of 40 STs, 19 were new to this study, but 16 of these new STs were within 11 known clonal complexes (257, 354, 21, 52, 353, 658, 45, 607, 61, 22 and 460). The most abundant newly described ST was ST-28. Identified 18 times, ST-28 is a single locus variant of ST-354, the central genotype of ST-354 (Mickan et al., 2007). A study from Michigan, USA of just 30 human and chicken isolates found 9 new STs, 5 of which were within established clonal complexes (Fitch et al., 2005). A Finnish study sequence subtyped 298 human isolates, from 1996, 2002 and 2003. Although 28 new STs were identified only 9 of these (each represented by just 1 isolate) were unassigned to existing clonal complexes, so that in total 264 of the 298 isolates were previously reported STs (Karenlampi et al., 2007); 25 were among the 19 new STs within known clonal complexes and just 9 were new STs and not members of known clonal complexes. A later UK study reported only clonal complexes of 326 isolates over a one-year period (2003/4). The main clonal complexes identified appear typical of earlier studies with no comment made on individual STs and no comparison with other datasets in the report (Sopwith et al., 2006). This study also noted a seasonal or transient peak in the summer of ST-45 complex, with the rise more marked in rural than urban areas. A sample from New Zealand in winter 2006, when rates of disease were unusually high identified just 2 new *C. jejuni* STs (4 isolates) in a sample of 107 isolates. Some groups of isolates within this study were unusual variants of common sequence subtypes e.g. ST-474 and ST-190 from clonal complexes ST-48 and 21 respectively. A common source local outbreak is suggested as the likely source (McTavish et al., 2007).

These results indicate geographical variation, but, more strikingly, that the same main clonal complexes dominate in human disease in much of the world. This is supported by later publications identifying STs that are already known or are minor variants of widely distributed STs identified in earlier studies in geographically distant areas. The precise ST profile present in each population (Duim et al., 2003; Mickan et al., 2007) and relative proportions of each ST (McTavish et al., 2007) varied somewhat between studies and suggests that there is some geographic structuring. Because several studies were of short duration, the extent to which such locally dominant STs represent stable geographical structuring or a transient expansion, perhaps due to a common source is not always clear (Duim et al., 2003; McTavish et al., 2007), but this uncertainty would be amenable to longitudinal sampling and subtyping or to the investigation of possible sources of transient expansions. Other expansions are clearly time limited, such as the ST-403 complex isolates in Curacao and ST-45 complex in North West England (Sopwith et al., 2006), which are consistent with either seasonal or temporary increases. As well as indexing short term variation these published studies form a geographically widespread baseline against which future studies using the same subtyping scheme can be compared to determine the longer term dynamics of population structure. The published work to date also shows the potential for more structured datasets to clarify questions of temporal-spatial variation in subtypes infecting humans and to use this to explore the origins of infection.

3.8.3. Subset analysis

A Finnish study used MLST to determine the sequence subtype of 46 isolates from an earlier case control study (Schonberg-Norio et al., 2004) and analysed risk factors by sequence subtype defined subgroups (Karenlampi et al., 2007). This identified some apparent associations, e.g. unassigned STs associated with swimming in natural bodies of water, eating raw mince with ST-48 complex, contact with a dog with ST-45 complex and drinking water from natural sources or untreated with chlorine with ST-677 complex. The sample sizes were small and analyses did not account for multiple testing statistically, which limits the certainty regarding the associations observed. Nonetheless, the findings are biologically plausible with, for example, the consumption of raw mince associated with a subtype most associated with ruminants and raw water consumption associated with a subtype typical of wild birds.

3.9. Summary of findings from the application of MLST

The direct comparison of studies using a standard MLST scheme allows substantially more extensive and robust inference than other subtyping methods, perhaps most clearly illustrated in reviewing host association and the geographical and temporal structure of human disease isolates. Attribution studies using model based analysis of MLST data are supportive of the majority, perhaps the large majority, of human infection originating in chicken but this is using very biased strain collections. However, questions remain in these areas and the value of more structured longitudinal samples, for example to allow separation of transient and seasonal effects, is evident as well as the need to validate attribution of human disease more fully. The limitations of MLST alone in supporting outbreak investigation highlight the importance of identifying effective complementary sequence based fine subtyping to develop the functions of supporting outbreak case definition and ideally to also allow outbreak detection. Such discriminatory fine subtyping should also support extension of the application to tracing infection through the food chain, where MLST has already started to be used. The results identified when MLST data was used in a small analytical epidemiology study support the integrated epidemiological analysis of classical risk factor information along with MLST subtype (Karenlampi et al., 2007). Alongside these more applied questions the demonstration of extensive recombination as contributing substantially to the patterns of MLST genotypes highlights that use of this data should consider this genetic process in any model based analysis and inference from subtyping data.

REFERENCES OF APPENDIX A

Allen VM, Bull SA, Corry JE, Domingue G, Jorgensen F, Frost JA, Whyte R, Gonzalez A, Elviss N and Humphrey TJ, 2007. *Campylobacter* spp. contamination of chicken carcasses during processing in relation to flock colonisation. Int J Food Microbiol, 113, 54-61.

Best EL, Fox AJ, Frost JA and Bolton FJ, 2004. Identification of *Campylobacter jejuni* multilocus sequence type ST-21 clonal complex by single-nucleotide polymorphism analysis. J Clin Microbiol, 42, 2836-9.

Best EL, Fox AJ, Frost JA and Bolton FJ, 2005. Real-time single-nucleotide polymorphism profiling using Taqman technology for rapid recognition of *Campylobacter jejuni* clonal complexes. J Med Microbiol, 54, 919-25.

Broman T, Palmgren H, Bergstrom S, Sellin M, Waldenstrom J, Danielsson-Tham ML and Olsen B, 2002. *Campylobacter jejuni* in black-headed gulls (*Larus ridibundus*): prevalence, genotypes, and influence on *C. jejuni* epidemiology. J Clin Microbiol, 40, 4594-602.

Broman T, Waldenstrom J, Dahlgren D, Carlsson I, Eliasson I and Olsen B, 2004. Diversities and similarities in PFGE profiles of *Campylobacter jejuni* isolated from migrating birds and humans. J Appl Microbiol, 96, 834-43.

Bull SA, Allen VM, Domingue G, Jorgensen F, Frost JA, Ure R, Whyte R, Tinker D, Corry JE, Gillard-King J and Humphrey TJ, 2006. Sources of *Campylobacter* spp. colonizing housed broiler flocks during rearing. Appl Environ Microbiol, 72, 645-52.

Champion OL, Gaunt MW, Gundogdu O, Elmi A, Witney AA, Hinds J, Dorrell N and Wren BW, 2005. Comparative phylogenomics of the food-borne pathogen *Campylobacter jejuni* reveals genetic markers predictive of infection source. Proc Natl Acad Sci USA, 102, 16043-8.

Chan K, Miller WG, Mandrell RE and Kathariou S, 2007. The absence of intervening sequences in 23S rRNA genes of *Campylobacter coli* isolates from Turkeys is a unique attribute of a cluster of related strains which also lack resistance to erythromycin. Appl Environ Microbiol, 73, 1208-14.

Clark CG, Beeston A, Bryden L, Wang G, Barton C, Cuff W, Gilmour MW and Ng LK, 2007. Phylogenetic relationships of *Campylobacter jejuni* based on *porA* sequences. Can J Microbiol, 53, 27-38.

Clark CG, Bryden L, Cuff WR, Johnson PL, Jamieson F, Ciebin B and Wang G, 2005. Use of the oxford multilocus sequence typing protocol and sequencing of the flagellin short variable region to characterize isolates from a large outbreak of waterborne *Campylobacter* spp. strains in Walkerton, Ontario, Canada. J Clin Microbiol, 43, 2080-91.

Colles FM, Jones K, Harding RM and Maiden MC, 2003. Genetic diversity of *Campylobacter jejuni* isolates from farm animals and the farm environment. Appl Environ Microbiol, 69, 7409-13.

Connerton PL, Loc Carrillo CM, Swift C, Dillon E, Scott A, Rees CE, Dodd CE, Frost J and Connerton IF, 2004. Longitudinal study of *Campylobacter jejuni* bacteriophages and their hosts from broiler chickens. Appl Environ Microbiol, 70, 3877-83.

D'Lima C B, Miller WG, Mandrell RE, Wright SL, Siletzky RM, Carver DK and Kathariou S, 2007. Clonal Population Structure and Specific Genotypes of Multidrug Resistant *Campylobacter coli* from Turkeys. Appl Environ Microbiol, 73, 2156-64.

Devane ML, Nicol C, Ball A, Klena JD, Scholes P, Hudson JA, Baker MG, Gilpin BJ, Garrett N and Savill MG, 2005. The occurrence of *Campylobacter* subtypes in environmental reservoirs and potential transmission routes. J Appl Microbiol, 98, 980-90.

Didelot X and Falush D, 2007. Inference of bacterial microevolution using multilocus sequence data. Genetics, 175, 1251-66.

Dingle KE, Colles FM, Falush D and Maiden MC, 2005. Sequence Typing and Comparison of Population Biology of *Campylobacter coli* and *Campylobacter jejuni*. J Clin Microbiol, 43, 340-7.

Dingle KE, Colles FM, Ure R, Wagenaar JA, Duim B, Bolton FJ, Fox AJ, Wareing DR and Maiden MC, 2002. Molecular characterization of *Campylobacter jejuni* clones: a basis for epidemiologic investigation. Emerg Infect Dis, 8, 949-55.

Dingle KE, Colles FM, Wareing DR, Ure R, Fox AJ, Bolton FE, Bootsma HJ, Willems RJ, Urwin R and Maiden MC, 2001a. Multilocus sequence typing system for *Campylobacter jejuni*. J Clin Microbiol, 39, 14-23.

Dingle KE, McCarthy ND, Cody AJ, Peto TE and Maiden MC, 2008. Extended sequence typing of *Campylobacter* spp., United Kingdom. Emerg Infect Dis, 14, 1620-2.

Dingle KE, Van Den Braak N, Colles FM, Price LJ, Woodward DL, Rodgers FG, Endtz HP, Van Belkum A and Maiden MC, 2001b. Sequence typing confirms that *Campylobacter jejuni* strains associated with Guillain-Barre and Miller-Fisher syndromes are of diverse genetic lineage, serotype, and flagella type. J Clin Microbiol, 39, 3346-9.

Djordjevic SP, Unicomb LE, Adamson PJ, Mickan L and Rios R, 2007. Clonal complexes of *Campylobacter jejuni* identified by multilocus sequence typing are reliably predicted by restriction fragment length polymorphism analyses of the *flaA* gene. J Clin Microbiol, 45, 102-8.

Duim B, Godschalk PC, van den Braak N, Dingle KE, Dijkstra JR, Leyde E, van der Plas J, Colles FM, Endtz HP, Wagenaar JA, Maiden MC and van Belkum A, 2003. Molecular evidence for dissemination of unique *Campylobacter jejuni* clones in Curacao, Netherlands Antilles. J Clin Microbiol, 41, 5593-7.

Duim B, Wassenaar TM, Rigter A and Wagenaar J, 1999. High-resolution genotyping of *Campylobacter* strains isolated from poultry and humans with amplified fragment length polymorphism fingerprinting. Appl Environ Microbiol, 65, 2369-75.

Duong T and Konkel ME, 2009. Comparative studies of *Campylobacter jejuni* genomic diversity reveal the importance of core and dispensable genes in the biology of this enigmatic food-borne pathogen. Current Opinion in Biotechnology, 20, 158-165.

Endtz HP, van West H, Godschalk PC, de Haan L, Halabi Y, van den Braak N, Kesztyus BI, Leyde E, Ott A, Verkooyen R, Price LJ, Woodward DL, Rodgers FG, Ang CW, van Koningsveld R, van Belkum A and Gerstenbluth I, 2003. Risk factors associated with *Campylobacter jejuni* infections in Curacao, Netherlands Antilles. J Clin Microbiol, 41, 5588-92.

Engberg J, Nachamkin I, Fussing V, McKhann GM, Griffin JW, Piffaretti JC, Nielsen EM and Gerner-Smidt P, 2001. Absence of clonality of *Campylobacter jejuni* in serotypes other than HS:19 associated with Guillain-Barré syndrome and gastroenteritis. J Infect Dis, 184, 215-20.

Evers EG, Van der Fels-Klerx HJ, Nauta MJ, Schijven JF and Havelaar AH, 2007. *Campylobacter* source attribution by exposure assessment. International Journal of Risk Assessment and Management, 8, 174-190.

Falush D, Stephens M and Pritchard JK, 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics, 164, 1567-87.

Fearnhead P, Smith NG, Barrigas M, Fox A and French N, 2005. Analysis of Recombination in *Campylobacter jejuni* from MLST Population Data. J Mol Evol, 61, 333-40.

Feavers IM, Gray SJ, Urwin R, Russell JE, Bygraves JA, Kaczmarski EB and Maiden MC, 1999. Multilocus sequence typing and antigen gene sequencing in the investigation of a meningococcal disease outbreak. J Clin Microbiol, 37, 3883-7.

Feil EJ, Li BC, Aanensen DM, Hanage WP and Spratt BG, 2004. eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. J Bacteriol, 186, 1518-30.

Fitch BR, Sachen KL, Wilder SR, Burg MA, Lacher DW, Khalife WT, Whittam TS and Young VB, 2005. Genetic diversity of *Campylobacter* spp. isolates from retail chicken products and humans with gastroenteritis in Central Michigan. J Clin Microbiol, 43, 4221-4.

Fitzgerald C, Helsel LO, Nicholson MA, Olsen SJ, Swerdlow DL, Flahart R, Sexton J and Fields PI, 2001. Evaluation of methods for subtyping *Campylobacter jejuni* during an outbreak involving a food handler. J Clin Microbiol, 39, 2386-90.

Fouts DE, Mongodin EF, Mandrell RE, Miller WG, Rasko DA, Ravel J, Brinkac LM, DeBoy RT, Parker CT, Daugherty SC, Dodson RJ, Durkin AS, Madupu R, Sullivan SA, Shetty JU, Ayodeji MA, Shvartsbeyn A, Schatz MC, Badger JH, Fraser CM and Nelson KE, 2005. Major structural differences and novel potential virulence mechanisms from the genomes of multiple *Campylobacter* species. PLoS Biol, 3, e15.

French N, Barrigas M, Brown P, Ribiero P, Williams N, Leatherbarrow H, Birtles R, Bolton E, Fearnhead P and Fox A, 2005. Spatial epidemiology and natural population structure of *Campylobacter jejuni* colonizing a farmland ecosystem. Environ Microbiol, 7, 1116-26.

Frost JA, Kramer JM and Gillanders SA, 1999. Phage typing of *Campylobacter jejuni* and *Campylobacter coli* and its use as an adjunct to serotyping. Epidemiol Infect, 123, 47-55.

Frost JA, Oza AN, Thwaites RT and Rowe B, 1998. Serotyping scheme for *Campylobacter jejuni* and *Campylobacter coli* based on direct agglutination of heat-stable antigens. J Clin Microbiol, 36, 335-9.

Garrett N, Devane ML, Hudson JA, Nicol C, Ball A, Klena JD, Scholes P, Baker MG, Gilpin BJ and Savill MG, 2007. Statistical comparison of *Campylobacter jejuni* subtypes from human cases and environmental sources. J Appl Microbiol, 103, 2113-21.

Gerner-Smidt P, Hise K, Kincaid J, Hunter S, Rolando S, Hyytia-Trees E, Ribot EM and Swaminathan B, 2006. PulseNet USA: a five-year update. Foodborne Pathog Dis, 3, 9-19.

Grajewski BA, Kusek JW and Gelfand HM, 1985. Development of a bacteriophage typing system for *Campylobacter jejuni* and *Campylobacter coli*. J Clin Microbiol, 22, 13-8.

Gressmann H, Linz B, Ghai R, Pleissner KP, Schlapbach R, Yamaoka Y, Kraft C, Suerbaum S, Meyer TF and Achtman M, 2005. Gain and Loss of Multiple Genes During the Evolution of *Helicobacter pylori*. PLoS Genet, 1, e43.

Hanninen ML, Hakkinen M and Rautelin H, 1999. Stability of related human and chicken *Campylobacter jejuni* genotypes after passage through chick intestine studied by pulsed-field gel electrophoresis. Appl Environ Microbiol, 65, 2272-5.

Hanninen ML, Perko-Makela P, Pitkala A and Rautelin H, 2000. A three-year study of *Campylobacter jejuni* genotypes in humans with domestically acquired infections and in chicken samples from the Helsinki area. J Clin Microbiol, 38, 1998-2000.

Harrington CS, Moran L, Ridley AM, Newell DG and Madden RH, 2003. Inter-laboratory evaluation of three flagellin PCR/RFLP methods for typing *Campylobacter jejuni* and *C. coli*: the CAMPYNET experience. J Appl Microbiol, 95, 1321-33.

Harrington CS, Thomson-Carter FM and Carter PE, 1997. Evidence for recombination in the flagellin locus of *Campylobacter jejuni*: implications for the flagellin gene typing scheme. J Clin Microbiol, 35, 2386-92.

Hedberg CW, Smith KE, Besser JM, Boxrud DJ, Hennessy TW, Bender JB, Anderson FA and Osterholm MT, 2001. Limitations of pulsed-field gel electrophoresis for the routine surveillance of *Campylobacter* infections. J Infect Dis, 184, 242-4.

Hepworth PJ, Leatherbarrow H, Hart CA and Winstanley C, 2007. Use of suppression subtractive hybridisation to extend our knowledge of genome diversity in *Campylobacter jejuni*. BMC Genomics, 8, 110.

Hook H, Ekegren MB, Ericsson H, Vagsholm I and Danielsson-Tham ML, 2004. Genetic and epidemiological relationships among *Campylobacter* isolates from humans. Scand J Infect Dis, 36, 435-42.

Hopkins KL, Desai M, Frost JA, Stanley J and Logan JM, 2004. Fluorescent amplified fragment length polymorphism genotyping of *Campylobacter jejuni* and *Campylobacter coli* strains and its relationship with host specificity, serotyping, and phage typing. J Clin Microbiol, 42, 229-35.

Johnsen G, Kruse H and Hofshagen M, 2006a. Genetic diversity and description of transmission routes for *Campylobacter* on broiler farms by amplified-fragment length polymorphism. J Appl Microbiol, 101, 1130-9.

Johnsen G, Kruse H and Hofshagen M, 2006b. Genotyping of *Campylobacter jejuni* from broiler carcasses and slaughterhouse environment by amplified fragment length polymorphism. Poult Sci, 85, 2278-84.

Johnsen G, Kruse H and Hofshagen M, 2007. Genotyping of thermotolerant *Campylobacter* from poultry slaughterhouse by amplified fragment length polymorphism. J Appl Microbiol, 103, 271-9.

Jolley KA, Chan MS and Maiden MC, 2004. mlstdbNet - distributed multi-locus sequence typing (MLST) databases. BMC Bioinformatics, 5, 86.

Karenlampi R, Rautelin H, Schonberg-Norio D, Paulin L and Hanninen ML, 2007. Longitudinal Study of Finnish *Campylobacter jejuni* and *C. coli* Isolates from Humans, Using Multilocus Sequence Typing, Including Comparison with Epidemiological Data and Isolates from Poultry and Cattle. Appl Environ Microbiol, 73, 148-55.

Keller J, Wieland B, Wittwer M, Stephan R and Perreten V, 2007. Distribution and genetic variability among *Campylobacter* spp. isolates from different animal species and humans in Switzerland. Zoonoses Public Health, 54, 2-7.

Kinana AD, Cardinale E, Bahsoun I, Tall F, Sire JM, Breurec S, Garin B, Saad-Bouh Boye C and Perrier-Gros-Claude JD, 2007. *Campylobacter coli* isolates derived from chickens in Senegal: Diversity, genetic exchange with *Campylobacter jejuni* and quinolone resistance. Res Microbiol, 158, 138-42.

Kinana AD, Cardinale E, Tall F, Bahsoun I, Sire JM, Garin B, Breurec S, Boye CS and Perrier-Gros-Claude JD, 2006. Genetic diversity and quinolone resistance in *Campylobacter jejuni* isolates from poultry in Senegal. Appl Environ Microbiol, 72, 3309-13.

Kuusi M, Nuorti JP, Hanninen ML, Koskela M, Jussila V, Kela E, Miettinen I and Ruutu P, 2005. A large outbreak of campylobacteriosis associated with a municipal water supply in Finland. Epidemiol Infect, 133, 593-601.

Lastovica AJ, Goddard EA and Argent AC, 1997. Guillain-Barré syndrome in South Africa associated with *Campylobacter jejuni* O:41 strains. J Infect Dis, 176 Suppl 2, S139-43.

Lior H, Woodward DL, Edgar JA, Laroche LJ and Gill P, 1982. Serotyping of *Campylobacter jejuni* by slide agglutination based on heat-labile antigenic factors. J Clin Microbiol, 15, 761-8.

Litrup E, Torpdahl M and Nielsen EM, 2007. Multilocus sequence typing performed on *Campylobacter coli* isolates from humans, broilers, pigs and cattle originating in Denmark. J Appl Microbiol, 103, 210-8.

Maiden MC, 2006. Multilocus sequence typing of bacteria. Annu Rev Microbiol, 60, 561-88.

Maiden MC, Bygraves JA, Feil E, Morelli G, Russell JE, Urwin R, Zhang Q, Zhou J, Zurth K, Caugant DA, Feavers IM, Achtman M and Spratt BG, 1998. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc Natl Acad Sci USA, 95, 3140-5.

Manning G, Dowson CG, Bagnall MC, Ahmed IH, West M and Newell DG, 2003. Multilocus sequence typing for comparison of veterinary and human isolates of *Campylobacter jejuni*. Appl Environ Microbiol, 69, 6370-9.

Manning G, Duim B, Wassenaar T, Wagenaar JA, Ridley A and Newell DG, 2001. Evidence for a genetically stable strain of *Campylobacter jejuni*. Appl Environ Microbiol, 67, 1185-9.

Martin IM, Ison CA, Aanensen DM, Fenton KA and Spratt BG, 2004. Rapid sequence-based identification of gonococcal transmission clusters in a large metropolitan area. J Infect Dis, 189, 1497-505.

McCarthy ND, Colles FM, Dingle KE, Bagnall MC, Manning G, Maiden MC and Falush D, 2007. Host-associated genetic import in *Campylobacter jejuni*. Emerg Infect Dis, 13, 267-72.

McTavish SM, Pope CE, Nicol C, Sexton K, French N and Carter PE, 2007. Wide geographical distribution of internationally rare *Campylobacter* clones within New Zealand. Epidemiol Infect, 1-9.

Meinersmann RJ, Dingle KE and Maiden MC, 2003. Genetic Exchange among *Campylobacter* Species. Genome Letters, 2, 48 - 52.

Meinersmann RJ, Helsel LO, Fields PI and Hiett KL, 1997. Discrimination of *Campylobacter jejuni* isolates by fla gene sequencing. J Clin Microbiol, 35, 2810-4.

Mellmann A, Mosters J, Bartelt E, Roggentin P, Ammon A, Friedrich AW, Karch H and Harmsen D, 2004. Sequence-Based Typing of *flaB* Is a More Stable Screening Tool Than Typing of *flaA* for Monitoring of *Campylobacter* Populations. J Clin Microbiol, 42, 4840-2.

Meyer M, Stenzel U and Hofreiter M, 2008. Parallel tagged sequencing on the 454 platform. Nat Protoc, 3, 267-78.

Michaud S, Menard S and Arbeit RD, 2005. Role of real-time molecular typing in the surveillance of *Campylobacter* enteritis and comparison of pulsed-field gel electrophoresis profiles from chicken and human isolates. J Clin Microbiol, 43, 1105-11.

Michaud S, Menard S, Gaudreau C and Arbeit RD, 2001. Comparison of SmaI-defined genotypes of *Campylobacter jejuni* examined by KpnI: a population-based study. J Med Microbiol, 50, 1075-81.

Mickan L, Doyle R, Valcanis M, Dingle KE, Unicomb L and Lanser J, 2007. Multilocus sequence typing of *Campylobacter jejuni* isolates from New South Wales, Australia. J Appl Microbiol, 102, 144-52.

Miller WG, Englen MD, Kathariou S, Wesley IV, Wang G, Pittenger-Alley L, Siletz RM, Muraoka W, Fedorka-Cray PJ and Mandrell RE, 2006. Identification of host-associated alleles by multilocus sequence typing of *Campylobacter coli* strains from food animals. Microbiology, 152, 245-55.

Miller WG, On SL, Wang G, Fontanoz S, Lastovica AJ and Mandrell RE, 2005. Extended multilocus sequence typing system for *Campylobacter coli*, *C. lari*, *C. upsaliensis*, and *C. helveticus*. J Clin Microbiol, 43, 2315-29.

Mishu B and Blaser MJ, 1993. Role of infection due to *Campylobacter jejuni* in the initiation of Guillain-Barré syndrome. Clin Infect Dis, 17, 104-8.

Nadeau E, Messier S and Quessy S, 2002. Prevalence and comparison of genetic profiles of *Campylobacter* strains isolated from poultry and sporadic cases of campylobacteriosis in humans. J Food Prot, 65, 73-8.

Neimann J, Engberg J, Molbak K and Wegener HC, 2003. A case-control study of risk factors for sporadic *Campylobacter* infections in Denmark. Epidemiol Infect, 130, 353-66.

O'Reilly LC, Inglis TJ and Unicomb L, 2006. Australian multicentre comparison of subtyping methods for the investigation of *Campylobacter infection*. Epidemiol Infect, 134, 768-79.

Ogden ID, MacRae M, Johnston M, Strachan NJ, Cody AJ, Dingle KE and Newell DG, 2007. Use of multilocus sequence typing to investigate the association between the presence of *Campylobacter* spp. in broiler drinking water and *Campylobacter* colonization in broilers. Appl Environ Microbiol, 73, 5125-9.

Parker CT, Miller WG, Horn ST and Lastovica AJ, 2007. Common genomic features of *Campylobacter jejuni* subsp. *doylei* strains distinguish them from *C. jejuni* subsp. *jejuni*. BMC Microbiol, 7, 50.

Parkhill J, Wren BW, Mungall K, Ketley JM, Churcher C, Basham D, Chillingworth T, Davies RM, Feltwell T, Holroyd S, Jagels K, Karlyshev AV, Moule S, Pallen MJ, Penn CW, Quail MA, Rajandream MA, Rutherford KM, van Vliet AH, Whitehead S and Barrell BG, 2000. The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. Nature, 403, 665-8.

Patton CM, Wachsmuth IK, Evins GM, Kiehlbauch JA, Plikaytis BD, Troup N, Tompkins L and Lior H, 1991. Evaluation of 10 methods to distinguish epidemic-associated *Campylobacter* strains. J Clin Microbiol, 29, 680-8.

Pearson AD, Greenwood MH, Feltham RK, Healing TD, Donaldson J, Jones DM and Colwell RR, 1996. Microbial ecology of *Campylobacter jejuni* in a United Kingdom chicken supply chain: intermittent common source, vertical transmission, and amplification by flock propagation. Appl Environ Microbiol, 62, 4614-20.

Penner JL and Hennessy JN, 1980. Passive hemagglutination technique for serotyping *Campylobacter fetus* subsp. *jejuni* on the basis of soluble heat-stable antigens. J Clin Microbiol, 12, 732-7.

Petersen L, Nielsen EM, Engberg J, On SL and Dietz HH, 2001. Comparison of genotypes and serotypes of *Campylobacter jejuni* isolated from Danish wild mammals and birds and from broiler flocks and humans. Appl Environ Microbiol, 67, 3115-21.

Price EP, Thiruvenkataswamy V, Mickan L, Unicomb L, Rios RE, Huygens F and Giffard PM, 2006. Genotyping of *Campylobacter jejuni* using seven single-nucleotide polymorphisms in combination with *flaA* short variable region sequencing. J Med Microbiol, 55, 1061-70.

Ragimbeau C, Salvat G, Colin P and Ermel G, 1998. Development of a multiplex PCR gene fingerprinting method using *gyrA* and *pflA* polymorphisms to identify genotypic relatedness within *Campylobacter jejuni* species. J Appl Microbiol, 85, 829-38.

Ribot EM, Fitzgerald C, Kubota K, Swaminathan B and Barrett TJ, 2001. Rapid pulsed-field gel electrophoresis protocol for subtyping of *Campylobacter jejuni*. J Clin Microbiol, 39, 1889-94.

Ridley AM, Toszeghy MJ, Cawthraw SA, Wassenaar TM and Newell DG, 2008. Genetic instability is associated with changes in the colonization potential of *Campylobacter jejuni* in the avian intestine. J Appl Microbiol, 105, 95-104.

Rosef O, Kapperud G, Lauwers S and Gondrosen B, 1985. Serotyping of *Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter laridis* from domestic and wild animals. Appl Environ Microbiol, 49, 1507-10.

Sails AD, Swaminathan B and Fields PI, 2003a. Clonal complexes of *Campylobacter jejuni* identified by multilocus sequence typing correlate with strain associations identified by multilocus enzyme electrophoresis. J Clin Microbiol, 41, 4058-67.

Sails AD, Swaminathan B and Fields PI, 2003b. Utility of multilocus sequence typing as an epidemiological tool for investigation of outbreaks of gastroenteritis caused by *Campylobacter jejuni*. J Clin Microbiol, 41, 4733-9.

Schonberg-Norio D, Sarna S, Hanninen ML, Katila ML, Kaukoranta SS and Rautelin H, 2006. Strain and host characteristics of *Campylobacter jejuni* infections in Finland. Clin Microbiol Infect, 12, 754-60.

Schonberg-Norio D, Takkinen J, Hanninen ML, Katila ML, Kaukoranta SS, Mattila L and Rautelin H, 2004. Swimming and *Campylobacter* infections. Emerg Infect Dis, 10, 1474-7.

Schouls LM, Reulen S, Duim B, Wagenaar JA, Willems RJ, Dingle KE, Colles FM and Van Embden JD, 2003. Comparative genotyping of *Campylobacter jejuni* by amplified fragment length polymorphism, multilocus sequence typing, and short repeat sequencing: strain diversity, host range, and recombination. J Clin Microbiol, 41, 15-26.

Scott AE, Timms AR, Connerton PL, Loc Carrillo C, Adzfa Radzum K and Connerton IF, 2007. Genome dynamics of *Campylobacter jejuni* in response to bacteriophage predation. PLoS Pathog, 3, e119.

Sheppard SK, Dallas JF, Strachan NJC, MacRae M, McCarthy ND, Wilson DJ, Gormley FJ, Falush D, Ogden ID, Maiden MCJ and Forbes KJ, 2009. *Campylobacter* Genotyping to Determine the Source of Human Infection. Clinical Infectious Diseases, 48, 1072–8.

Siemer BL, Harrington CS, Nielsen EM, Borck B, Nielsen NL, Engberg J and On SL, 2004. Genetic relatedness among *Campylobacter jejuni* serotyped isolates of diverse origin as determined by numerical analysis of amplified fragment length polymorphism (AFLP) profiles. J Appl Microbiol, 96, 795-802.

Smith JM, Smith NH, O'Rourke M and Spratt BG, 1993. How clonal are bacteria? Proc Natl Acad Sci USA, 90, 4384-8.

Sopwith W, Ashton M, Frost JA, Tocque K, O'Brien S, Regan M and Syed Q, 2003. Enhanced surveillance of *Campylobacter* infection in the North West of England 1997-1999. J Infect, 46, 35-45.

Sopwith W, Birtles A, Matthews M, Fox A, Gee S, Painter M, Regan M, Syed Q and Bolton E, 2006. *Campylobacter jejuni* multilocus sequence types in humans, northwest England, 2003-2004. Emerg Infect Dis, 12, 1500-7.

Suerbaum S, Lohrengel M, Sonnevend A, Ruberg F and Kist M, 2001. Allelic diversity and recombination in *Campylobacter jejuni*. J Bacteriol, 183, 2553-9.

Suzuki Y, Ishihara M, Saito M, Ishikawa N and Yokochi T, 1994. Discrimination by means of pulsed-field gel electrophoresis between strains of *Campylobacter jejuni* Lior type 4 derived from sporadic cases and from outbreaks of infection. J Infect, 29, 183-7.

Thakur S and Gebreyes WA, 2005. *Campylobacter coli* in swine production: antimicrobial resistance mechanisms and molecular epidemiology. J Clin Microbiol, 43, 5705-14.

Thakur S, Morrow WE, Funk JA, Bahnson PB and Gebreyes WA, 2006. Molecular epidemiologic investigation of *Campylobacter coli* in swine production systems, using multilocus sequence typing. Appl Environ Microbiol, 72, 5666-9.

Wareing DR, Ure R, Colles FM, Bolton FJ, Fox AJ, Maiden MC and Dingle KE, 2003. Reference isolates for the clonal complexes of *Campylobacter jejuni*. Lett Appl Microbiol, 36, 106-10.

Wassenaar TM and Newell DG, 2000. Genotyping of *Campylobacter* spp. Appl Environ Microbiol, 66, 1-9.

Wieland B, Wittwer M, Regula G, Wassenaar TM, Burnens AP, Keller J and Stark KD, 2006. Phenon cluster analysis as a method to investigate epidemiological relatedness between sources of *Campylobacter jejuni*. J Appl Microbiol, 100, 316-24.

Wilson DJ, Gabriel E, Leatherbarrow AJ, Cheesbrough J, Gee S, Bolton E, Fox A, Fearnhead P, Hart CA and Diggle PJ, 2008. Tracing the source of campylobacteriosis. PLoS Genet, 4, e1000203.

Yan W, Chang N and Taylor DE, 1991. Pulsed-field gel electrophoresis of *Campylobacter jejuni* and *Campylobacter coli* genomic DNA and its epidemiologic application. J Infect Dis, 163, 1068-72.

Zhang Q, Meitzler JC, Huang S and Morishita T, 2000. Sequence polymorphism, predicted secondary structures, and surface-exposed conformational epitopes of *Campylobacter* major outer membrane protein. Infect Immun, 68, 5679-89.

B. MODELLING ATTRIBUTION APPROACHES BASED ON MICROBIAL SUBTYPING

The five current mathematical models that have been reported in the literature are described below.

1. The Dutch Model

The Dutch Model (French and the Molecular Epidemiology and Veterinary Public Health Group, 2008; Van Pelt et al., 2006) is a straight forward way to estimate the attribution of a particular genotype (e.g. sequence subtype (ST)) to a reservoir, when the frequency distribution of each subtype is known for each reservoir. If p_{ij} represents the frequency of subtype *i* (e.g. ST 19) in source *j* (e.g. poultry) then the expected number of cases of subtype *i* from source *j* is given by

$$\lambda_{ij} = \frac{p_{ij}}{\sum_j p_{ij}} x_i$$

Where x_i is the estimated number of human cases of subtype *i*, the summation by *j* considers all the reservoirs where data exist (e.g. cattle, sheep, wild birds, poultry etc.).

When applied at the ST level this model does not guarantee that all STs will be attributed to sources. This is because human subtypes, which are not found in the animal reservoir, cannot be attributed. However, if genetic information exists at multiple loci (e.g. 7 loci for this study), then the Dutch Model can make use of the frequency of each individual allele at each individual loci, and estimate attribution even for STs which are not present in the animal reservoirs. In particular, at the allele level the frequencies $p_{a_{ijk}}$ can be calculated for each allele a_{ijk} of all isolates from the animal reservoirs. Where i is subtype, j the source and k the loci number (see Table 1).

Table 1

Loci1	Loci2	Loci3	Loci4	Loci5	Loci6	Loci7	MLST subtype
a_{1j1}	a_{1j2}					a_{1j7}	1
a_{ij1}	a_{ij2}					a_{ij7}	i

The attribution score of bacterial subtype *i* in source *j* is

$$\lambda_{ij} = \frac{\prod_{k=1}^{7} p_{a_{ijk}}}{\sum_j \left(\prod_{k=1}^{7} p_{a_{ijk}} \right)} x_i$$

where $p_{a_{ijk}} = BetaInv(0.5, 0+1, N_{isolates}+1)$ if its frequency is zero (*BetaInv* fn from Excel alternately in more usual notation Beta(α,β) where α=1, β=$N_{isolates}$+1). This assumes that we have no prior knowledge of $p_{a_{ijk}}$ and so is maximally non-commital or conservative.

2. Population structure

STRUCTURE (Pritchard et al., 2000) is a Bayesian clustering model designed to infer population structure and to attribute individuals to population groups. The program readily uses MLST genotyping data. Each isolate is attributed on the basis of a training dataset consisting of isolates from known populations (i.e. set USEPOPINFO to 1). The algorithm calculates the frequency of each particular sequence subtype in each population taking into account the uncertainty due to the sample size. Based on these frequencies the probability of belonging to a population group/reservoir is calculated, following multiple iterative steps (MARKOV CHAIN MONTE CARLO- MCMC) for the estimation of frequencies. The program has the option to consider the allele independent (no-admixture model – independent alleles) and start with equal frequencies for each subtype. Following an initial number of MCMC burn-in steps (e.g. 1,000) further itterations (e.g. 10,000) are used for estimation of the probabilities that an isolate belongs to each particular population being considered (e.g cattle, sheep, poultry etc.). To enable the largest reference dataset to be used (often datasets are small due to the cost of subtyping many isolates) only one ST is selected at a time from the unknown dataset using the jacknife method. This process is repeated to enable multiple estimations of the same ST so that uncertainty in the attribution scores can be determined.

3. Hald Model

A more advanced application of the microbial subtyping approach was developed in Denmark for the attribution of human salmonellosis (Hald et al., 2004). Using data from the integrated Danish *Salmonella* surveillance programme, a mathematical model was developed to quantify the contribution of each of the major food animal sources to human *Salmonella* infections. The "Danish *Salmonella* source attribution" model uses a Bayesian framework with Markov Chain Monte Carlo simulation to attribute sporadic laboratory-confirmed human *Salmonella* infections caused by different *Salmonella* subtypes as a function of the prevalence of these subtypes in animal and food sources and the amount of each food source consumed.

The estimated number of cases for each serotype is considered to be Poisson distributed with the expected/average value being dependent on source, exposure (amount of food consumed, prevalence of bacteria, dose) and bacteria-dependant factors (e.g. survivability, virulence, pathogenicity).

Briefly, the number of human cases of subtype *i* is

$$o_i \propto Poisson\left(\sum_j \lambda_{ij}\right)$$

where

$$\lambda_{ij} = M_j p_{ij} q_i a_j$$

λ_{ij} represents the expected number of human cases of subtype *i* from source *j*.

A description of the parameters of Hald Model is given in Table 2.

Table 2: Description of parameters in the Hald Model.

Parameters	Description
λ_{ij}	Expected number of cases/year of subtype *i* from source *j*
M_j	Amount of food source *j* consumed
p_{ij}	Prevalence of subtype *i* in source *j*
q_i	Bacteria-dependent factor for subtype *i*
a_j	Source-dependent factor for source *j*
o_i	Number of human cases of subtype *i*

The model takes into account the uncertainty for all these factors and also includes travel as a possible risk factor.

4. Modified Hald Model

This model is a variation of the Hald Model that has been developed for use with both *Salmonella* and *Campylobacter* (Mullner et al., 2009). The modifications include: removal of the food factor variables, incorporation of the uncertainty in prevalence of the source reservoirs and incorporation of environmental sources.

4.1. Modelling the uncertainty of prevalence in animal and environmental sources

The prevalence uncertainty is modelled using beta priors in the risk model,

$$p_{ij} \propto Beta(\alpha_{ij}, \beta_{ij}).$$

The factors α_{ij} and β_{ij} are determined in a separate Bayesian model, where three datasets are used to model the prevalence: overall prevalence data (all untyped positive and negative *Campylobacter*), typed positive and negative, and typed positive only. The frequencies of each subtype and the overall prevalence are incorporated into a Likelihood function, which is maximized starting with independent Dirichlet priors for frequencies, and independent beta priors for overall prevalence. The average and standard deviation for prevalence p_{ij} is obtained and α_{ij} and β_{ij} are chosen to fit this distribution.

4.2. Modelling the bacteria dependent factor q_i

In the Hald Model some of the q_i's are assumed to be equal to ensure that there are fewer unknown variables than observed data points.

In the modified Hald model the variability in virulence is included in a lognormal distribution using a unique parameter, τ.

Briefly,

$$\log(q_i) \propto N(0, \tau)$$

where the prior for τ is chosen by a diffuse gamma function, $\tau \propto Gamma(0.01, 0.01)$.

4.3. Modelling the source specific parameters a_j

The source specific parameter – is a measure of the concentration of bacteria in a particular source (e.g. bacteria/kg). This is modelled using independent exponential priors.

This prevents a_j being too large and hence an upper limit does not need to be specified as in the Hald Model.

4.4. Avoidance of food consumption weights

These are removed (M_j in the Hald model) and in effect are absorbed into the source-specific parameter a_j. This means that exposure to a specific amount from a specific source and subtype is not explicitly modelled.

5. Asymmetric island (AI) model

The Asymmetric Island Model (Wilson et al., 2008) incorporates a Bayesian approach and uses the allelic profile of the sequence subtypes to reconstruct the genealogical history of the isolates. The host populations are considered to exist on separate "islands" (e.g. the island sheep). Mutations and recombination occur on each island. Migrations from each reservoir (island) into the human population are used to estimate the degree of attribution to each source.

The model has two stages:

- ***the evolutionary stage*** – estimates the rates of mutation, recombination and migration of *Campylobacter* host populations (is complex, uses an approximate likelihood function):
    - does not involve *Campylobacter* from humans;
    - samples by Markov Chain Monet Carlo (MCMC) techniques from the posterior distribution of the rates to be used in the second stage;
- ***the attribution stage*** – human isolates are considered random samples from the reservoir populations; is used to estimate the posterior probability of attribution to each source.

REFERENCES OF APPENDIX B

French NP and the Molecular Epidemiology and Veterinary Public Health Group HRI, 2008. Enhancing Surveillance of Potentially Foodborne Enteric Diseases in New Zealand: Human Campylobacteriosis in the Manawatu. Final report: FDI / 236 /2005.

Hald T, Vose D, Wegener HC and Koupeev T, 2004. A Bayesian approach to quantify the contribution of animal-food sources to human salmonellosis. Risk Anal, 24, 255-69.

Mullner P, Jones G, Noble A, Spencer SEF, Hathaway S and French NP, 2009. Source Attribution of Food-Borne Zoonoses in New Zealand: A Modified Hald Model. Risk Analysis, 29, 970-984.

Pritchard JK, Stephens M and Donnelly P, 2000. Inference of population structure using multilocus genotype data. Genetics, 155, 945-59.

Van Pelt W, Van de Giessen AW, Mevius DJ, Vennema H, Koopmans M, Van Asten L and van Duynhoven YTHP, 2006. Trends in gastro-enteritis van 1996-2005. Toename van ziekenhuisopnames en sterfte: een toenemende rol.van virale infecties? Infectieziekten Bulletin, 17, 364-369.

Wilson DJ, Gabriel E, Leatherbarrow AJ, Cheesbrough J, Gee S, Bolton E, Fox A, Fearnhead P, Hart CA and Diggle PJ, 2008. Tracing the source of campylobacteriosis. PLoS Genet, 4, e1000203.

C. METHODOLOGIES FOR SOURCE ATTRIBUTION OF HUMAN CAMPYLOBACTERIOSIS BY EPIDEMIOLOGICAL APPROACHES

1. Source attribution of human campylobacteriosis by a systematic review of case-control studies as reported in section 5.1. of the opinion

1.1. Methodology used

The formal process for literature review established for systematic reviews (SR) was followed (Sargeant et al., 2006). The objective was to investigate the most important risk factors for human sporadic campylobacteriosis, and the process included (1) literature search, (2) identification of relevant literature, (3) quality assessment of relevant studies, (4) summarization and statistical analysis of data, and (5) conclusions. Citations were collected, de-duplicated and managed in a web-based software (SRS 4.0, TrialStat! 93 Corporation, Ottawa, ON). The meta-analyses consisted of the analysis of the summarized statistics of the studies provided by the SR. Collected information corresponded to the number of cases and controls exposed and not-exposed to extracted risk factors, and an estimated odds ratio and the 95% confidence interval for the same risk factors. All extracted risk factors were stratified according to defined source-categorizations schemes. An overall meta-analysis was conducted within each risk factor stratum if estimates were available from more than two studies. The Cochran-Mantel-Haenszel test for SAS was utilized to assess the homogeneity between studies and calculate a common pooled odds ratio and 95% confidence interval, using the number of cases and controls exposed and non-exposed. To estimate these values, the studies were weighted according the number of cases/controls enrolled in the study (Anonymous, 2004). Following the initial meta-analysis, a set of sensitivity analyses to evaluate the impact of the inclusion of studies from different regions in the world, time periods and age groups was performed. The same methodology was followed, but applied to sub-sets of the data, according to the above categories. All analyses were performed in SAS Enterprise Guide, SAS Insitute., SAS/STAT® User's Guide, Version 8, Cary, NC: SAS Institute Inc., 1999.

1.2. List of collected case-control studies of sporadic campylobacteriosis

From 1,295 identified references, 132 passed the relevant screening, 73 passed the quality assessment stage, and data was extracted from 72. From these, 37 investigated risk factors of sporadic campylobacteriosis.

A list of all collected case-control studies of sporadic campylobacteriosis is reported in Table 1.

Table 1: List of collected case-control studies.

Reference	Country	Region	Time period	Number of Cases	Number of Controls
Wingstrand et al., 2006	Denmark	Northern EU	2000-2001	107	178
Ethelberg et al., 2005	Denmark	Northern EU	1991-2001	22,066	318,958
Carrique-Mas et al., 2005	Sweden	Northern EU	2001-2002	126	270
Schonberg-Norio et al., 2004	Finland	Northern EU	2002 (Summer)	100	137
Kapperud et al., 2003	Norway	Northern EU	1999-2000	212	422
Tenkate and Stafford, 2001	Australia	Oceania	1996-1997	81	144
Rodrigues et al., 2001	UK	Northern Europe	1995-1996	229	229
Studahl and Andersson, 2000	Sweden	Northern EU	1995	101	198
Effler et al., 2001	US	North America	1998 (May-Sept.)	211	211
Neal and Slack, 1997	UK	Northern EU	1994-1995	531	512
Eberhart-Phillips et al., 1997	New Zealand	Oceania	1994-1995	621	621
Adak et al., 1995	UK	Northern EU	1990-1991	598	738
Schorr et al., 1994	Switzerland	Western EU	1991	167	282
Kapperud et al., 1992a	Norway	Northern EU	1989-1990	58	117
Hudson et al., 1991	UK	Northern EU	1990 (April-June)	30	30
Grados et al., 1988	Peru	South America	1983-1986	104	104
Deming et al., 1987	US	North America	1983-1984	45	45
Stafford et al., 2007	Australia	Oceania	2001-2002	881	833
Michaud et al., 2004	Canada	North America	2000-2001	158	314
Cameron et al., 2004	Australia	Oceania	2000-2001	172	169
Neimann et al., 2003	Denmark	Northern EU	1996-1997	282	319
Neal and Slack, 1995	UK	Northern EU	1983-1984	245	247
Schmid et al., 1987	US	North America	1982-1983		
Harris et al., 1986	US	North America	1982-1983	218	526
Lighton et al., 1991	UK	Northern EU	1990 (May-June)	29	41
Fullerton et al., 2007	US	North America	2002-2004	123	928
Bellido-Blasco et al., 2007	Spain	Southern EU	2000	117	84
Kassenborg et al., 2004	US	North America	1998-1999	64	?
Friedman et al., 2004	US	North America	1998-1999	1,316	1,316
Potter et al., 2003	US	North America	2000-2001	83	122
Unicomb et al., 2008	Australia	Oceania	1981 (Summer)	354	593
Hopkins et al., 1984	US	North America	1981	40	71
Kapperud et al., 1992b	Norway	Northern EU	1989-1990	52	103
Hauge et al., 1996	Norway	Northern EU	1991-1994	56	117
Gallay et al., 2008	France	Western EU	2002-2004	285	286
Stafford et al., 2008	Australia	Oceania	2001-2002	881	883

2. Source attribution of human campylobacteriosis by an analysis of data from outbreak investigations as reported in section 5.2. of the opinion

2.1. Methodology used for attribution of illnesses due to complex foods

Food items were categorized in a hierarchical scheme (see Figure 1). Foods that contained ingredients that are members of a single commodity were considered ''simple foods,'' while foods that contained ingredients that are members of multiple commodities were considered ''complex foods''. Each implicated food was assigned to one or more mutually exclusive food commodities, according to its ingredients. Illnesses in an outbreak due to a complex food item were attributed to a commodity in the implicated complex food, only if that commodity had been implicated in at least one outbreak due to a simple food. As an example, outbreak-associated illnesses caused by *lasagna* would be attributed to the commodities dairy, beef, vegetables, grains and beans, and oils and sugar. If any of these commodities was not implicated in any outbreak caused by simple foods, the commodity would be excluded from the calculations for the attribution of illnesses to the ingredients composing the complex food. For the remaining commodities, the proportion of illnesses within the outbreak attributed to each commodity was estimated based on the number of illnesses caused by the commodity in simple foods-outbreaks and the sum of illnesses caused by all the commodities that compose the food. For outbreaks caused by complex foods where ingredients were unavailable, these were provided by a review of recipes on the web, as described by Painter (2006): the top three recipes from a World Wide Web (Google) search were selected; when recipes were conflicted, the ingredients listed in at least two of the three recipes were included.

The two separate analyses were performed in an attempt to account for potential overestimations of the proportion of disease attributed to sources that caused large outbreaks. For the purpose of source attribution of human disease, only the proportion of disease attributable to sources, as estimated in the analysis by the number of outbreaks, was used. The estimated proportions were multiplied by the total number of laboratory-confirmed *Campylobacter* infections reported in the study period to estimate the number of cases attributable to each source.

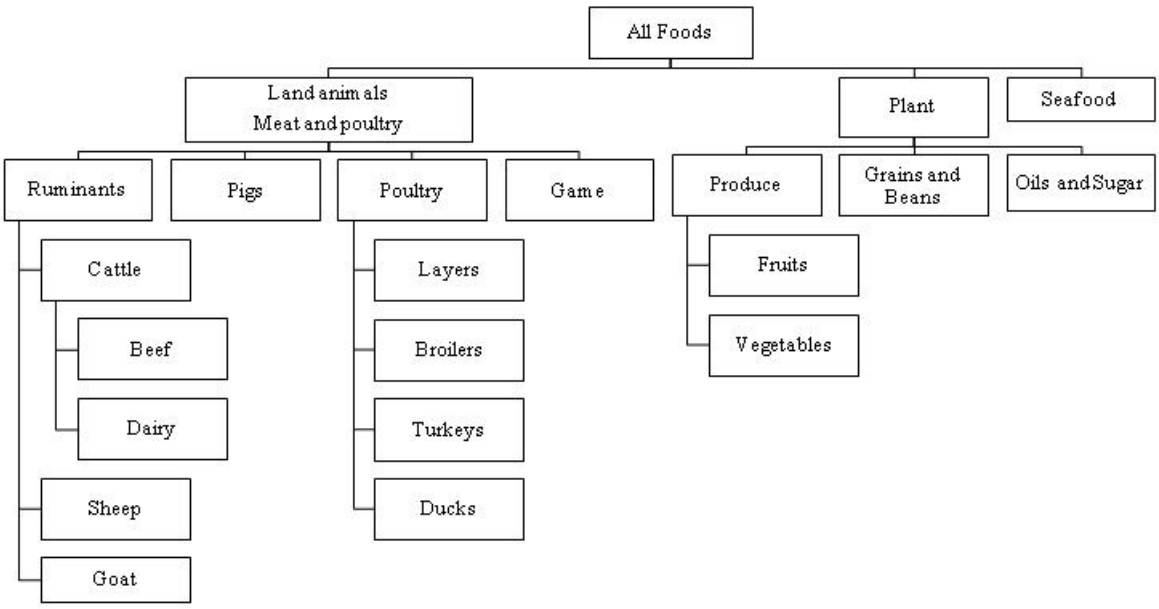

Figure 1: Hierarchical scheme for categorisation of foods (Pires, 2009).

REFERENCES OF APPENDIX C

Adak GK, Cowden JM, Nicholas S, Evans HS, 1995. The Public Health Laboratory Service national case-control study of primary indigenous sporadic cases of *Campylobacter* infection. Epidemiol. Infect. 115, 15-22.

Anonymous, 2004. SAS Institute Inc. 2004 SAS/STAT 9.1 User's Guide. Cary, NC: SAS Institute Inc. 2004.

Bellido-Blasco JB, Gonzalez-Cano JM, Galiano-Arlandis JV, Herrero-Carot C, Tirado-Balaguer MD, Arnedo-Pena A, Safont-Adsuara L, Romeu-Garcia MA, 2007. Risk factors for the occurrence of sporadic *Campylobacter*, *Salmonella* and rotavirus diarrhea in preschool children. An. Pediatr. (Barc.) 66, 367-374.

Cameron S, Ried K, Worsley A, Topping D, 2004. Consumption of foods by young children with diagnosed *Campylobacter* infection - a pilot case-control study. Public Health Nutr. 7, 85-89.

Carrique-Mas J, Andersson Y, Hjertqvist M, Svensson A, Torner A, Giesecke J, 2005. Risk factors for domestic sporadic campylobacteriosis among young children in Sweden. Scand. J. Infect. Dis. 37, 101-110.

Deming MS, Tauxe RV, Blake PA, Dixon SE, Fowler BS, Jones TS, Lockamy EA, Patton CM, Sikes RO, 1987. *Campylobacter* enteritis at a university: transmission from eating chicken and from cats. Am. J. Epidemiol. 126, 526-534.

Eberhart-Phillips J, Walker N, Garrett N, Bell D, Sinclair D, Rainger W, Bates M, 1997. Campylobacteriosis in New Zealand: results of a case-control study. J. Epidemiol. Community Health 51, 686-691.

Effler P, Ieong MC, Kimura A, Nakata M, Burr R, Cremer E, Slutsker L, 2001. Sporadic *Campylobacter jejuni* infections in Hawaii: associations with prior antibiotic use and commercially prepared chicken. J. Infect. Dis. 183, 1152-1155.

Ethelberg S, Simonsen J, Gerner-Smidt P, Olsen KE, Molbak K, 2005. Spatial distribution and registry-based case-control analysis of *Campylobacter* infections in Denmark, 1991-2001. Am. J. Epidemiol. 162, 1008-1015.

Friedman CR, Hoekstra RM, Samuel M, Marcus R, Bender J, Shiferaw B, Reddy S, Ahuja SD, Helfrick DL, Hardnett F, Carter M, Anderson B, Tauxe RV, 2004. Risk factors for sporadic *Campylobacter* infection in the United States: A case-control study in FoodNet sites. Clin. Infect. Dis. 38 Suppl 3, S285-S296.

Fullerton, K.E., Ingram, L.A., Jones, T.F., Anderson, B.J., McCarthy, P.V., Hurd, S., Shiferaw, B., Vugia, D., Haubert, N., Hayes, T., Wedel, S., Scallan, E., Henao, O., Angulo, F.J., 2007. Sporadic *Campylobacter* infection in infants: a population-based surveillance case-control study. Pediatr. Infect. Dis. J. 26, 19-24.

Gallay A, Bousquet V, Siret V, Prouzet-Mauleon V, Valk H, Vaillant V, Simon F, Le Strat Y, Megraud F and Desenclos J C, 2008. Risk factors for acquiring sporadic *Campylobacter* infection in France: results from a national case-control study. *J Infect Dis* 197. 10, 1477-84.

Grados O, Bravo N, Black RE, Butzler JP, 1988. Paediatric *Campylobacter* diarrhoea from household exposure to live chickens in Lima, Peru. Bull. World Health Organ 66, 369-374.

Harris NV, Weiss NS, Nolan CM, 1986. The role of poultry and meats in the etiology of *Campylobacter jejuni*/*coli* enteritis. Am. J. Public Health 76, 407-411.

Hauge K, 1996. Risk factors for sporadic *Campylobacter* infection. Results from a case-control study in Trøndelag, Central Norway, Project report for the degree of Master of Science, London School of Hygiene and Tropical Medicine, Department of Public Health and Policy.

Hopkins RS, Olmsted R, Istre GR, 1984. Endemic *Campylobacter jejuni* infection in Colorado: identified risk factors. Am. J. Public Health 74, 249-250.

Hudson SJ, Lightfoot NF, Coulson JC, Russell K, Sisson PR, Sobo AO, 1991. Jackdaws and magpies as vectors of milkborne human *Campylobacter* infection. Epidemiol. Infect. 107, 363-372.

Kapperud G, Espeland G, Wahl E, Walde A, Herikstad H, Gustavsen S, Tveit I, Natas O, Bevanger L, Digranes A, 2003. Factors associated with increased and decreased risk of *Campylobacter* infection: a prospective case-control study in Norway. Am. J. Epidemiol. 158, 234-242.

Kapperud G, Lassen J, Ostroff SM, Aasen S, 1992a. Clinical features of sporadic *Campylobacter* infections in Norway. Scand. J. Infect. Dis. 24, 741-749.

Kapperud G, Skjerve E, Bean NH, Ostroff SM, Lassen J, 1992b. Risk factors for sporadic *Campylobacter* infections: results of a case-control study in southeastern Norway. J. Clin. Microbiol. 30, 3117-3121.

Kassenborg HD, Smith KE, Vugia DJ, Rabatsky-Ehr T, Bates MR, Carter MA, Dumas NB, Cassidy MP, Marano N, Tauxe RV, Angulo FJ, 2004. Fluoroquinolone-resistant *Campylobacter* infections: eating poultry outside of the home and foreign travel are risk factors. Clin. Infect. Dis. 38 Suppl 3, S279-S284.

Lighton LL, Kaczmarski EB, Jones DM, 1991. A study of risk factors for *Campylobacter* infection in late spring. Public Health 105, 199-203.

Michaud S, Menard S, Arbeit RD, 2004. Campylobacteriosis, Eastern Townships, Quebec. Emerg. Infect. Dis. 10, 1844-1847.

Neal KR, Slack RC, 1997. Diabetes mellitus, anti-secretory drugs and other risk factors for *Campylobacter* gastro-enteritis in adults: a case-control study. Epidemiol. Infect. 119, 307-311.

Neal KR, Slack RC, 1995. The autumn peak in *Campylobacter* gastro-enteritis. Are the risk factors the same for travel- and UK-acquired *Campylobacter* infections? J. Public Health Med 17, 98-102.

Neimann J, Engberg J, Molbak K, Wegener HC, 2003. A case-control study of risk factors for sporadic *Campylobacter* infections in Denmark. Epidemiol. Infect. 130, 353-366.

Painter J, 2006. Estimating attribution of illnesses to food vehicle from reports of foodborne outbreak investigations. Society for for Risk Analysis, 2006 Annual Meeting. 3-6 December 2006, Baltimore, MD.

Pires SM, 2009. Attributing human salmonellosis and campylobacteriosis to animal, food and environmental sources. PhD thesis. ISBN 978-87-7611-311-7. University of Copenhagen, Faculty of Life Sciences, Copenhagen.

Potter RC, Kaneene JB, Hall WN, 2003. Risk factors for sporadic *Campylobacter jejuni* infections in rural Michigan: a prospective case-control study. Am. J. Public Health 93, 2118-2123.

Rodrigues LC, Cowden JM, Wheeler JG, Sethi D, Wall PG, Cumberland P, Tompkins DS, Hudson MJ, Roberts JA, Roderick PJ, 2001. The study of infectious intestinal disease in England: risk factors for cases of infectious intestinal disease with *Campylobacter jejuni* infection. Epidemiol. Infect. 127, 185-193.

Sargeant JM, Rajic A, Read S and Ohlsson A, 2006. The process of systematic review and its application in agri-food public-health. *Preventive Veterinary Medicine* 75. 3-4, 141-151.

Schmid GP, Schaefer RE, Plikaytis BD, Schaefer JR, Bryner JH, Wintermeyer LA, Kaufmann AF, 1987. A one-year study of endemic campylobacteriosis in a midwestern city: association with consumption of raw milk. J. Infect. Dis. 156, 218-222.

Schonberg-Norio D, Takkinen J, Hanninen ML, Katila ML, Kaukoranta SS, Mattila L, Rautelin H, 2004. Swimming and *Campylobacter* infections. Emerg. Infect. Dis. 10, 1474-1477.

Schorr D, Schmid H, Rieder HL, Baumgartner A, Vorkauf H, Burnens A, 1994. Risk factors for *Campylobacter* enteritis in Switzerland. Zentralbl. Hyg. Umweltmed. 196, 327-337.

Stafford RJ, Schluter P, Kirk M, Wilson A, Unicomb L, Ashbolt R, Gregory J, 2007. A multi-centre prospective case-control study of *Campylobacter* infection in persons aged 5 years and older in Australia. Epidemiol. Infect. 135, 978-988.

Stafford RJ, Schluter PJ, Wilson AJ, Kirk MD, Hall G, Unicomb L, 2008. Population-attributable risk estimates for risk factors associated with *Campylobacter* infection, australia. Emerg. Infect. Dis. 14, 895-901.

Studahl A, Andersson Y, 2000. Risk factors for indigenous *Campylobacter* infection: a Swedish case-control study. Epidemiol. Infect. 125, 269-275.

Tenkate TD, Stafford RJ, 2001. Risk factors for *Campylobacter* infection in infants and young children: a matched case-control study. Epidemiol. Infect. 127, 399-404.

Unicomb LE, Dalton CB, Gilbert GL, Becker NG, Patel MS, 2008. Age-specific risk factors for sporadic *Campylobacter* infection in regional Australia. Foodborne Pathog. Dis. 5, 79-85.

Wingstrand A, Neimann J, Engberg J, Nielsen EM, Gerner-Smidt P, Wegener HC, Molbak K, 2006. Fresh chicken as main risk factor for campylobacteriosis, Denmark. Emerg. Infect. Dis. 12, 280-285.

GLOSSARY

Case-control study	The observational epidemiologic study of persons with the disease of interest and a suitable control group of persons without the disease. The relationship of an attribute to the disease is examined by comparing the diseased and non-diseased with regard to how frequently the attribute is present (Last, 1995).
Case-series study	A group or series of case reports involving patients who were given similar treatment (for the purposes of this document, "those patients who have the same clinical symptoms"). Reports of case series usually contain detailed information about the individual patients. This includes demographic information (for example, age, gender, ethnic origin) and information on diagnosis, treatment, response to treatment, and follow-up after treatment[18].
Cohort study (syn. Longitudinal study)	The analytic method of epidemiologic study in which subsets of a defined population can be defined who are, or have been, or in the future may be exposed or not exposed, or exposed to different degrees, to a factor or factors hypothesized to influence the probability of occurrence of a given disease or other outcome (Last, 1995).
Food-borne outbreak	Incidence, observed under given circumstances, of two or more human cases of the same disease and/or infection, or a situation in which the observed number of cases exceeds the expected number and where the cases are linked, or are probably linked, to the same food source (EC, 2003).
Human illness source attribution	Partitioning of the human disease burden to one or more specific sources.
Intervention study	A study involving intentional change in some aspect of the status of subjects, e.g. introduction of a preventive or therapeutic regimen, or designed to test a hypothesized relationship (Last, 1995).
Points of attribution	Points in the food chain where human illness source attribution can take place, including production, distribution and consumption.
Reservoir	An animate (humans, animals, insects etc.) or inanimate object (plant, soil, etc.) or any combination of these serving as a habitat of a pathogen that produces itself in such a way as to be transmitted to a susceptible host (Toma et al., 1999).
Source	Origin of the pathogen causing infection, including reservoirs, vectors and vehicles.
Sporadic case	Case that has not been associated with known outbreaks (Engberg, 2006).

18 National Cancer Institute. Dictionary of Cancer Terms. http://www.cancer.gov/Templates/db_alpha.aspx?CdrID=44006 (accessed on 1 December 2009).

Vector	A living organism which, because of its ecological relationship to others, acquires a pathogen from one living host, and transmits it to another (Toma et al., 1999).
Vehicle	An object, a substance, or non-receptive living being serving as an intermediary in transmitting a pathogen from the organism hosting it to a receptive host (Toma et al., 1999).

REFERENCES OF GLOSSARY

EC, 2003. Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC (OJ L 325, 12.12.2003, p. 31).

Engberg J, 2006. Contributions to the epidemiology of *Campylobacter* infections. A review of clinical and microbiological studies. Dan Med Bull 53, 4, 361-89.

Last JM (ed), 1995. A dictionary of epidemiology. Third edition. New York, Oxford University Press.

Toma B, Vaillancourt JP, Dufour B, Eloit M, Moutou F, Marsh W, Benet JJ, Sanaa M and Michel P, 1999. Dictionary of veterinary epidemiology. IOWA State University Press.