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The Increasing Incidence of Human Campylobacteriosis. Report and Proccedings of a WHO Consultation of Experts

Copenhagen, Denmark
21-25 November 2000

World Health Organization
Department of Communicable Disease Surveillance and Response

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The Increasing Incidence of Human Campylobacteriosis

Report and Proceedings of a WHO Consultation of Experts

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Department of Communicable Disease Surveillance and Response
World Health Organization

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Table of Contents

1. Executive summary 4

2. Introduction 6

3. General conclusions and recommendations 7
3.1 Conclusions 7
3.2 Recommendations 7

4. Health impact of *Campylobacter* infections 9
4.1 Morbidity 9
4.2 Mortality 9
4.3 Sequelae 10
4.4 The burden of *Campylobacter* species other than *C. jejuni* and *C. coli* . . .10
4.5 Antibiotic resistance 11

5. Epidemiology of *Campylobacter* 12
5.1 *Campylobacter* infection rates 12
5.2 Pathways for transmitting *Campylobacter* 13
5.3 Identification of risk factors 13
5.4 *Campylobacter* types in foods and reservoirs 14
5.5 Outbreak investigations 14
5.6 Intervention studies 14

6. Development of laboratory infrastructure 16
6.1 Establishment of regional and national reference facilities 16
6.2 Laboratory guidelines for *Campylobacter* 16
6.3 Education and training programmes 18
6.4 Technology transfer 18
6.5 Development and standardization of laboratory methods 19

7. Surveillance of *Campylobacter* and campylobacteriosis 20
7.1 Surveillance in developed countries 20
7.2 Developing countries 21
7.3 Non human reservoirs of *Campylobacter* 21

8. Intervention and control of *Campylobacter* 23
8.1 Developing countries 23
8.2 Developed countries 23
8.3 Specific control measures 24
8.3. Poultry 24
8.3.2 Biosecurity in poultry production 25
8.3.3 Biosecurity for other food animals 25
8.3.4 Use of antibiotics in food animals 26
8.3.5 Travel 26
8.3.6 Food preparation and handling 26
8.3.7 Processing 27
8.3.8 End-product decontamination by irradiation 28

Table 1. 29

Table 2. 29

Annex 1 Programme of the consultation 30

Annex 2 List of participants 31

Annex 3 working papers 35

Annex 4 Country Reports 116

1. Executive summary

The public health burden of campylobacteriosis is increasing. The reported incidence of campylobacteriosis in most developed countries has risen substantially during the past 20 years, and especially since 1990. In developing countries, campylobacteriosis is widespread and causes significant morbidity, and even mortality, in infants and children. Additional concerns are raised by the number of newly described *Campylobacter* species, as well as the increasing number of antibiotic-resistant strains of the common species, *C. jejuni*. Recently, too, it has been recognized that the paralytic condition, Guillain-Barr Syndrome (GBS), is a serious complication of *Campylobacter* infections.

Many risk factors for *Campylobacter* transmission have been identified. In developed countries, for example, handling and consumption of poultry meat are often causes of infection and are likely to account for much of the increased incidence of campylobacteriosis. Other risk factors include foods of animal origin, including raw milk, inadequately treated water, contact with farm animals and pets, and foreign travel. Many transmission pathways vary in importance with time and location.

Despite the increasing importance of campylobacteriosis, most developing countries, and even many developed countries, do not have surveillance systems to measure the health and economic burden of human campylobacteriosis, nor detect trends in outbreaks. Few countries, for example, survey animals and other foodstuffs for *Campylobacter*, which hampers investigation into the sources and routes of *Campylobacter* transmission. In many countries, too, the capacity to detect and respond to outbreaks of campylobacteriosis is also insufficient.

In light of these conclusions, it is recommended that WHO take a leading role in informing national governments, public health officials, food producers, retailers and the general public worldwide about the importance of *Campylobacter* in enteric disease. National governments, for example, can be encouraged to recognize the public health importance of *Campylobacter* by ranking it with *Salmonella* and *Shigella* as a leading enteric bacterial pathogen. It is also recommended that the true public health burden and social consequences of campylobacteriosis and its sequelae be determined in both developing and developed countries.

To help achieve these goals, ongoing surveillance systems for *Campylobacter* and campylobacteriosis should be developed. These systems should be able to monitor trends over time and seasons, and measure the effects of public health controls and interventions. Surveillance programmes for animals and food, for example, should identify sources and routes of human *Campylobacter* transmission, and provide baseline data for intervention. In developing countries, every paediatric faecal sample examined for *Salmonella* and *Shigella* should also be examined for *Campylobacter*; this strategy should be adopted for all patients with diarrhoeic disease in developed countries. All *Campylobacter* isolates from developed countries should be tested for antibiotic resistance.

To support surveillance and control programme, countries need to develop strong laboratory infrastructures. Appropriately equipped laboratories are fundamental to effective enteric disease control programmes, including those for controlling

Campylobacter. In the developing world, existing regional laboratory centres of excellence should be strengthened, and new centres established. Ideally, the laboratories should have the capacity for culturing and typing pathogens, and for testing pathogen sensitivity to antibiotics. A strong laboratory infrastructure will also help to develop a set of guidelines for characterizing pathogens, including *Campylobacter*, and form part of regional and national networks of reference facilities. Training could be provided for individuals in developing countries, and their laboratory and surveillance skills improved, if national/regional laboratory centres collaborated with centres in the developed world.

2. Introduction

Campylobacter is the leading cause of zoonotic enteric infections in developed and developing countries, and the incidence is increasing even in countries with adequate public health surveillance. Campylobacteriosis has severe, but rare, sequelae: Guillian-Barr Syndrome (a severe paralytic condition), arthritis, and septicaemia in immunocompromised populations such as AIDS patients. Campylobacteriosis and its sequelae are under-recognized because of inadequate national surveillance, because few outbreaks occur and because the infections rarely are fatal. This partly explains why activities to investigate, survey and control *Campylobacter* and campylobacteriosis are often underrepresented when compared to efforts to control infections caused by other zoonotic enteric pathogens, such as *Salmonella* and enteropathogenic *E. col*i (EHEC).

Campylobacter is the leading cause of zoonotic enteric infections in developed and developing countries.

Many risk factors for C*ampylobacter* exist, in line with its ubiquitous occurrence in food production, wild fauna and in the environment. Although our understanding of the risk factors for the infection has improved in recent years, the relative contribution of the different factors to the total incidence of disease has not been established, and this may partly explain why few targeted control programmes exist. Recently, however, a few countries launched new initiatives to reduce *Campylobacter* in food products (Denmark and Iceland) and to reduce the presence of fluoroquinolone-resistant *Campylobacter* in food (USA).

To address this issue, WHO invited 29 experts on *Campylobacter* and campylobacteriosis to a meeting on The Increasing Incidence of Campylobacteriosis in Copenhagen, Denmark, 20-25 November 2000. The meeting objectives were to:

- ¥ Review data on the incidence and trends of campylobacteriosis in developed and developing countries.
- ¥ Provide an overview of current knowledge on the epidemiology of campylobacteriosis.
- ¥ Review existing control programmes.
- ¥ Identify gaps in our knowledge of campylobacteriosis epidemiology, and to produce recommendations on the needs of basic and applied research on the epidemiology, surveillance and control of *Campylobacter* and campylobacteriosis.
- ¥ Strengthen international collaboration between key people involved in *Campylobacter* research and control.

¥ Support the development and harmonization of laboratory diagnostic tools.
¥ Strengthen surveillance of *Campylobacter* and campylobacteriosis in developed and developing countries.

3. General conclusions and recommendations

3.1 Conclusions

¥ ***The public health burden of campylobacteriosis is increasing.*** The reported incidence of campylobacteriosis in most developed countries has risen substantially during the past 20 years, and especially since 1990. In developing countries, campylobacteriosis is widespread and causes significant morbidity, and even mortality, in infants and children. Additional concerns are raised by the number of newly described *Campylobacter* species, as well as the increasing number of antibiotic-resistant strains of the common species, *C. jejuni.* Recently, too, it has been recognized that the paralytic condition, Guillain-Barr Syndrome (GBS), is a serious complication of *Campylobacter* infections.

¥ ***Enteric disease surveillance systems are inadequate.*** In most developing countries, and even in many developed countries, surveillance systems cannot determine the burden of human campylobacteriosis, nor detect trends in outbreaks. Few countries, for example, survey animal and other foodstuffs for *Campylobacter*, which hampers investigation of the sources and routes of *Campylobacter* transmission. In many countries, too, the capacity to detect and respond to outbreaks of campylobacteriosis is also insufficient.

¥ ***Many risk factors for Campylobacter transmission have been identified.*** In developed countries, for example, handling and consumption of poultry meat are primary sources of infection and are likely to account for much of the increased incidence of campylobacteriosis. Other risk factors in developed countries include foods of animal origin, including raw milk, inadequately treated water, contact with farm animals and pets, and foreign travel. In developing countries, inadequately treated water and contact with farm animals are assumed to be the most important risk factors. The significance of different transmission pathways varies with time and location.

¥ ***There is insufficient knowledge of control measures.*** The current knowledge as to the most appropriate measures for controlling the different sources of transmission is limited. Generation of such knowledge is essential to support the establishment of priorities for control in developed and developing countries.

3.2 Recommendations

- WHO should take a leading role in informing national governments, public health officials, food producers, retailers and the general public worldwide, about the importance of *Campylobacter* in enteric disease.

- The public health burden and social consequences of campylobacteriosis and its sequelae should be determined in both developing and developed countries.

- National governments should ensure that *Campylobacter* is ranked with *Salmonella* and *Shigella* as leading enteric bacterial pathogens, to reflect its public health importance.

- Reporting systems for *Campylobacter* and campylobacteriosis should be developed. Surveillance should be ongoing and monitor trends over time and seasons, as well as changes following public health control and intervention measures. Surveillance programmes for animals and food should identify sources and routes of human *Campylobacter* transmission, and provide baseline data for intervention.

- In developing countries, every paediatric faecal sample examined for *Salmonella* and *Shigella* should also be examined for *Campylobacter*. In developed countries this strategy should be adopted for all patients with diarrhoeic disease. All isolates from developed countries should be tested for antibiotic resistance.

- Countries need to develop strong laboratory infrastructures to counter the lack of surveillance and response capacity. In the developing world, existing regional laboratory centres of excellence should be strengthened and new centres established. Appropriately equipped laboratories are fundamental to effective enteric disease control programmes, including those for controlling *Campylobacter*. Ideally, the laboratories should have the capacity for culturing and typing pathogens, and for testing pathogen sensitivity to antibiotics. A strong laboratory infrastructure will also help in developing a set of guidelines for characterizing pathogens, including *Campylobacter*, and form part of regional and national networks of reference facilities. Collaboration with centres in the developed world would provide laboratory and surveillance skills and training and should be supported.

Countries need to develop strong laboratory infrastructures to counter the lack of surveillance and response capacity.

4. Health impact of *Campylobacter* infections

4.1 Morbidity

Current state

The reported incidence of campylobacteriosis has risen substantially in many developed countries during the past 20 years, especially since 1990, and the increase is too large to be explained solely by changes in medical care, laboratory practices, or disease reporting. This suggests that the disease itself is occurring more frequently.

The true incidence of campylobacteriosis in countries such as the USA and the UK is uncertain, since many unreported infections occur for every diagnosed case, but it may exceed one percent per year. In developing countries the disease burden is not known, but is likely to be high.

Needs

- To measure diarrhoeal illness directly in the population and to measure the proportion attributable to *Campylobacter*.

- To define the multipliers for estimating the number of cases in a population from a small number of reported cases. This will improve the accuracy of trends measured within countries, and estimates of disease incidence between countries could be more readily compared.

4.2 Mortality

Current state

A recent analysis of *Campylobacter* mortality at 30 days post-infection estimated the case-fatality rate to be as high as 4 deaths per 1000 infections. However, this figure may underestimate the true fatality rate, since *Campylobacter* can cause death a year or more following infection. HIV infection may also contribute to the mortality rate following *Campylobacter* infections. Obtaining accurate measurements of mortality is important since small errors in these measurements lead to large inaccuracies when extrapolated to the health burden in populations.

Needs

- To improve estimates of mortality using linked registry data or other novel approaches.
- To ascertain the importance of HIV-related mortality in *Campylobacter* cases in the developing world, especially in areas with high HIV prevalence.

4.3 Sequelae

With the eradication of polio, GBS may be the most common cause of acute flaccid paralysis (AFP).

Current state

Acute diarrhoeal disease is the most frequent result of *Campylobacter* infection, but the organism has also been implicated in a variety of other clinical conditions, many of which are rare and have limited public health significance. For example, it has recently been recognized that the paralytic condition, Guillain-Barr Syndrome, is a serious complication of *Campylobacter* infections. GBS cases associated with *Campylobacter* infection are usually more severe and can require intensive hospital treatment and long-term disability. With the eradication of polio, GBS may be the most common cause of acute flaccid paralysis (AFP). *Campylobacter* infection is also associated with reactive arthritis, but the public health significance is less well understood. Available epidemiological and surveillance data on acute disease and sequelae can be integrated in health burden assessments (e.g. using Disability Adjusted Life Years); and conducting economic assessments would better represent the impact of campylobacteriosis on society.

Needs

- ¥ To measure the burden of GBS associated with *Campylobacter* infection, including the long-term consequences of GBS.
- ¥ To assess the bacteriological and host factors that lead to GBS, and to estimate how many GBS cases can be prevented by reducing the number of *Campylobacter* infections.
- ¥ To identify GBS cases by expanding existing surveillance for AFP.
- ¥ To describe the association between *Campylobacter* infection and reactive arthritis, using a standardized case definition, and to describe other long-term sequelae.

4.4 The burden of *Campylobacter* species other than *C. jejuni* and *C. coli*

Current state

The role of *Campylobacter* species other than C. jejuni and C. coli in clinical disease is unclear. Not all laboratories use methods that detect these other species, many of which have only recently been described, and controlled studies of the clinical and public health significance of them have not been conducted. Although C. upsaliensis is recognized as a pathogen in children in some countries, data from different parts of the world can only be compared quantitatively when comparable methods are used. The role of other species, such as C. consisus, in clinical disease is more doubtful. Differences in the frequency of *Campylobacter* species in different geographical areas need to be confirmed.

Needs

- ¥ To determine the clinical relevance of *Campylobacter* species other than *C. jejuni* and *C. coli* in different geographical areas and in different patient groups.

4.5 Antibiotic resistance

Current state

The resistance of *Campylobacter* to antibiotics is increasing. Except for a temporary increase in erythromycin resistance, the most alarming increase in resistance is to the fluoroquinolone group of antimicrobials. This is because adult patients suffering from severe gastrointestinal disease are likely to be treated with a fluoroquinolone without prior confirmation of the diagnosis, and if the strain of *Campylobacter* is fluoroquinolone resistant, the duration of the disease may be prolonged. One of the major reasons for the increase of fluoroquinolone-resistant strains in humans is the use of these antibiotics in poultry. Seasonality in the incidence of the fluoroquinolone-resistant strains has also been reported in different countries and the use of this epidemiological marker may allow the frequency of fluoroquinolone-resistant strains to be quantified at different locations during the year.

The resistance of Campylobacter to antibiotics is increasing.

Needs

- ¥ A better evaluation of the clinical impact of antibiotic-resistant strains of *Campylobacter*.
- ¥ To determine the sources of antibiotic-resistant strains of *Campylobacter*.
- ¥ To study how the changing use of antibiotics in animals affects the emergence or disappearance of resistant *Campylobacter* strains.

5. Epidemiology of *Campylobacter*

The public health burden of *Campylobacter* will likely increase in the future, because new species of *Campylobacter* are being discovered and an increasing number of antibiotic-resistant strains are appearing in the common species, *C. jejuni.* Epidemiological findings can be used to help address this burden by influencing policy in national programmes. For example, *Campylobacter* can be transmitted to humans by several pathways, which vary in importance with time and location. Demonstrating that specific interventions limit *Campylobacter* transmission via particular pathways can be an important step in translating epidemiological findings to full national programmes.

Needs

- ¥ Continued support for ongoing surveillance, plus additional support for direct population measures of diarrhoeal illness.
- ¥ Measurements of the GBS burden associated with *Campylobacter* infection.
- ¥ An assessment of the pathogenic potential of newly described *Campylobacter* species.
- ¥ More information on the clinical impact of antibiotic-resistant strains of *Campylobacter* and their reservoirs.
- ¥ More accurate estimates of the disease burdens of *Campylobacter* reservoirs.
- ¥ To promote and encourage intervention studies.
- ¥ To promote the integration of strain typing and epidemiological information.

5.1 *Campylobacter* infection rates

Current state

Campylobacter infections increase sharply in early summer, persist through the fall and decrease in winter. This seasonal pattern repeats with little variation within countries. But between countries, the time of onset, duration and peak incidence of infection can all vary significantly. *Campylobacter* infection is particularly common in young children and young adults, and affects more men than women. Often, it is more common among city dwellers than rural populations. In the developing world, campylobacteriosis is more common among young children, and it is presumed that older persons have acquired immunity following repeated exposures.

Needs

- ¥ A better understanding of the causes underlying the seasonal variation of *Campylobacter* infections, including the roles played by animals and the environment.
- ¥ An explanation of why more males become infected with *Campylobacter* than females.
- ¥ A serological assay to measure seroprevalence and to study the relationship between seropositivity and disease protection.

5.2 Pathways for transmitting *Campylobacter*

Current state

Campylobacter can be transmitted by several routes, which can be considered as strata. The depth and relative importance of each stratum varies with time and location, and their quantification is not straightforward. For example, cross contamination means that identified food vehicles may differ from the original sources of contamination. In developed countries, poultry is often considered to be a primary source of infection and is likely to account for many of the increased number of campylobacteriosis cases seen in recent years. In developing countries, by contrast, waterborne transmission or direct animal-to-man transmission may be common because of poor sanitation and because people live in close association with food animals. Waterborne transmission can also cause sporadic outbreaks in developed countries, and even large outbreaks if there is a failure of routine water treatment. Other potential sources of infection include raw milk and contact with pets or other animals; direct infection from one person to another can occur, but is uncommon. Many cases of campylobacteriosis are associated with foreign travel, from 10-50% or more of all cases depending on the country, and usually result from the consumption of contaminated food or water in the countries visited.

In developing countries, by contrast, waterborne transmission or direct animal-to-man transmission may be common because of poor sanitation and because people live in close association with food animals.

Needs

- The contributions of *Campylobacter* reservoirs to disease outbreaks need to be more accurately evaluated, including the role of non-poultry meat and other food sources.
- The role of cross-contamination in restaurants and homes needs to be quantified.

5.3 Identification of risk factors

Current state

Case-control studies of sporadic cases provide useful information about specific risk factors for *Campylobacter* infection. Several case-control studies have been carried out. Most studies have identified poultry products as important risk factors. However, it has been difficult to establish the number of cases directly attributable to poultry, because the risk of infection from contaminated poultry strongly depends on hygiene practices in the kitchen, which are difficult to assess. Other risk factors include the consumption of meats other than poultry at restaurants or barbecues, drinking untreated water or raw milk, contact with animals, and travel. Protective factors include eating chicken or other meat prepared at home and eating chicken purchased frozen. Many of the studies were limited in size and may not have identified all common risk factors. It is unknown to what extent acquired immunity among control subjects influenced the results of case-control studies. The inclusion of strain typing in case-control studies may identify type-specific sources of infection.

Needs

- Targeted case-control studies with hypotheses and objectives built on previous experiences.
- Cooperation between groups planning and evaluating case-control studies.

¥ An assessment of the effect of the immune status of controls on case-control studies, using a standardized serological method.

5.4 *Campylobacter* types in foods and reservoirs

Current state

Typing of *Campylobacter* strains is a powerful tool for tracing associations among strains separated in time or by location, and it has been used to track the flow of strains through the food chain and to investigate outbreaks. Typing may also prove useful for monitoring trends over time and for monitoring case-control studies. However, the relationship between *Campylobacter* types obtained from human populations and those from foods or other possible sources of contamination should be evaluated with caution. In general, it is important to combine epidemiological and microbiological information when assessing typing studies.

Needs

¥ To assess whether *Campylobacter* typing in human disease surveillance is a useful public health intervention.

¥ Collaboration between microbiologists and epidemiologists in the design and interpretation of typing surveys of different *Campylobacter* reservoirs.

¥ To evaluate type-specific variation in clinical cases of campylobacteriosis and in transmission pathways.

5.5 Outbreak investigations

Current state

It is important to investigate outbreaks of campylobacteriosis promptly and thoroughly. Outbreak investigations provide detailed information about the sources of human infection and about routes of infection, and also raise the public profile of campylobacteriosis. The role of *Campylobacter* in waterborne outbreaks may be underrecognized.

Needs

¥ A systematic investigation of the exposed population and long-term sequelae during large outbreaks of campylobacteriosis. Outbreak investigations also offer a unique opportunity to assess the role of immunity and of exposure doses.

¥ To investigate whether *Campylobacter* is responsible for outbreaks of waterborne diarrhoea.

5.6 Intervention studies

Current state

Intervention studies that limit *Campylobacter* transmission via particular pathways provide powerful epidemiological evidence of the importance of the pathways in transmission, especially when combined with systematic surveillance, and can be an important step in translating epidemiological findings to full national programmes. For example, a simple intervention trial in Bolivian families showed that disinfecting the stored drinking water reduced all diarrhoeal illness by 44% and

Campylobacter diarrhoea by more than 80%. In Iceland, the incidence of domestically acquired human *Campylobacter* infections decreased in 2000, following on-farm intervention in poultry rearing in which birds from *Campylobacter*-positive flocks were slaughtered and frozen separately. If the effect is sustained, this intervention model may be useful for designing future intervention methods. Regulatory changes, such as a change in freezing or cooking requirements; or unusual events, such as the recent temporary ban on poultry consumption in Belgium because of dioxin concerns, may offer unique opportunities to measure the effect of targeted interventions.

Needs

- To take advantage of regulatory changes or emergency responses to study the efficacy of targeted interventions.

6. Development of laboratory infrastructure

To be effective, programmes for controlling enteric diseases need the support of a strong laboratory infrastructure; this is true for both developing and developed countries. The ability to diagnose, perform surveillance and develop control strategies for *Campylobacter* is crucial in this respect, but unfortunately many countries are either unable to carry out these functions, or have only limited ability to do so. In the following sections, 6.1-6.5, we discuss recommendations for strengthening laboratory infrastructures for *Campylobacter* programmes.

To be effective, programmes for controlling enteric diseases need the support of a strong laboratory infrastructure.

6.1 Establishment of regional and national reference facilities

Developing countries: Existing facilities should be identified and reviewed, and reference centres established accordingly. Efforts should also be made to establish information networks that link local, regional and national reference centres. Whenever possible, the networks should include human clinical, veterinary, food and environmental agencies, as appropriate. Local laboratories should also include *Campylobacter* in their enteric disease programmes.

Developed countries: It is recommended that networks of clinical, veterinary, food and environmental agencies be established, to facilitate the rapid transfer and evaluation of data critical for protecting public health. In many locations, however, it is still not routine to check for *Campylobacter* and reference facilities are not available. In such cases, the above recommendations for developing countries apply.

6.2 Laboratory guidelines for Campylobacter

Clinical samples: Samples to be used in the bacteriological diagnosis of enteric infection should be delivered to the laboratory and processed as quickly as possible. Transport medium should be used for stool (preferred), or rectal swab samples if a delay greater than four hours is anticipated. Modern blood culture systems are efficient at detecting *Campylobacter* bacteriaemia and blood cultures should be taken if the clinical features and immunocompetence of the patient indicate campylobacteriosis.
Isolation protocol: In developed countries, most *Campylobacter* infections in

humans are caused by C. jejuni and *C. coli*, and culture methods for these organisms are well established. Evidence is accumulating, however, indicating that other *Campylobacter* species may be clinically relevant and multiple species can be present in clinical samples. Unfortunately, the optimal culture conditions for other *Campylobacter* species are not known. Current recommendations for their culture can be found in Table 1 and Table 2. Further studies to determine the incidence, reservoirs and clinical significance of these organisms are therefore essential.

Food, water and animals: Food, water and animal specimens should be examined as part of targeted surveillance programmes. When working with *C. jejuni* and *C. coli* microbiologists should use established national or international standard methods, published by agencies such as ISO, FDA and NMKL. Quantitative methods are needed to aid risk assessments and to evaluate the success of intervention measures in the food chain. Such methods include most probable number techniques and surface count methods. Methods for transporting food, water, animal, environmental and other samples for examination require further development.

Identification: The minimal standards for identifying *Campylobacter* after primary isolation are: colony morphology, Gram stain response, motility and an oxidase test. The hippurate hydrolysis test differentiates most C. jejuni strains from other *Campylobacter* species. For organisms other than *C. jejuni* and *C. coli*, including atypical *C. jejuni* strains, additional biochemical tests are required. Several molecular tests can further characterise the strains, and the introduction of molecular techniques in developing countries is encouraged. However, these tests require a laboratory and there would need to be suitable technology transfer procedures to ensure their success.

To assist in the epidemiological identification of *Campylobacter*, a simple, reliable and inexpensive method is needed for storing reference strains and isolates long-term. Identification schema should be regularly reviewed to account for new taxonomic developments.

Non-culture methods: Traditional microscopic methods, such as using Gram stain response and cell motility, are useful for examining fresh, acute phase clinical specimens. Recently, commercial antigen detection kits have become available for detecting *C. jejuni* and *C. coli*. Molecular methods, based on the polymerase chain reaction (PCR), have also been developed for identifying several *Campylobacter* species. Antigen and molecular based methods need to be validated, however, before they are used extensively to diagnose and survey *Campylobacter* infection.

Serodiagnosis: Available immunodiagnostic assays are neither standardized nor validated, but they are necessary to study the aetiology of postinfective sequelae, such as GBS, and to provide estimates of protective immunity for epidemiological studies. Optimized assays should be developed which can also be used with noninvasive clinical samples, such as saliva.

Typing Methods: Typing methods should be implemented as a critical component for epidemiological studies in both developed and developing countries. Methods should be selected according to the objective, e.g. local outbreak assessment or long-term surveillance, and the available resources. The typing strategy should be developed in consultation with epidemiologists. A wide range of typing methods is

available and should be applied to the epidemiological analysis, as appropriate. Efforts are underway to standardize certain genotyping methods in Europe (www.svs.dk/campynet) and in the USA (PulseNet: www.cdc.gov/ncidod/dbmd/pulsenet/pulsenet.htm). The selection of a standard typing method will facilitate international comparison and institutes should be encouraged to cooperate in its development. In particular, there is a critical need for a typing method that relates *Campylobacter* types to pathogenic potential. A better understanding of the factors that cause virulence in *Campylobacter* strains is also needed, as are methods for detecting them.

Antimicrobial susceptibility testing: For patient treatment, antimicrobial susceptibility testing may be indicated if it is clinically relevant. Although testing is an important component of surveillance studies, standardized tests are urgently required. MIC assays (i.e. an E-test) and disk diffusion assays for nalidixic acid would be suitable. There is a need to develop simple, reliable and inexpensive assays to address the lack of information on resistance patterns.

Quality Control: Quality control procedures must be established as part of the overall laboratory programme. The best way of doing this would be to establish regional reference laboratories.

6.3 Education and training programmes

In developed countries, key laboratories should participate in training and education programmes that include both practical and theoretical courses on the clinical importance, diagnosis, treatment and epidemiology of campylobacteriosis. The laboratories should be adequately funded and established *Campylobacter* reference laboratories should organize the courses, provide guidelines and materials, and ensure quality control.

In developing countries, facilities for education and training already exist in some regions and should be enhanced. Initially, fellowships should be awarded to experienced scientists (preferably at doctoral level) to organize practical and theoretical training courses in all enteropathogens at established reference centres. Financial resources should also be made available to organize local facilities, and the activities of the local centres should be monitored by a system of quality control and regular reporting. Periodic training updates should also be ensured. The local centres should organize regional training and education courses where necessary in cooperation with experts from developed countries. To assist with this, contacts with international organisations should be stimulated, and selected staff should be supported for training and education in developed countries.

The Internet has emerged as an important information and educational tool and should be used more extensively to disseminate information about *Campylobacter*. Current initiatives, such as Global Salm-Surv and CampyNet could serve as models for the development of future programmes. In addition, support for scientists to attend international scientific meetings, should be encouraged since this will make them more aware of current research.

The Internet should be used more extensively to disseminate information about Campylobacter.

6.4 Technology transfer

Once an effective reference laboratory and training structure for basic methods has been established, further improvements will require the transfer of more advanced technologies, such as PCR, molecular typing and sequencing techniques. To this end, collaboration and exchange between developing and developed countries should be stimulated. An essential part of this will be the creation of national/regional/global information networks, as well as making equipment, consumables and technical support available in a timely manner. Local or regional companies need to be involved or established to provide maintenance and repair and to supply parts and consumables. Research into the standardization and application of laboratory methods for *Campylobacter* should also be encouraged.

6.5 Development and standardization of laboratory methods

To improve knowledge of human campylobacteriosis and to make optimal use of funding, collaborative research between national and international groups should be promoted. Developed nations could make *Campylobacter* research more accessible to developing countries by providing technological and financial support, and local scientists in developing countries should be encouraged to set up collaboration with researchers in developed countries. In particular, research that addresses local needs in developing countries should be promoted.

7. Surveillance of *Campylobacter* and campylobacteriosis

Surveillance provides data, which can help guide plans for action. Baseline data, for example, assess pre-intervention conditions, which helps determine whether intervention is required. If intervention is necessary, the effects of the programme can then be measured against the baseline data.

7.1 Surveillance in developed countries

Current state

Although many developed countries have laboratory-based surveillance systems that usually include *Campylobacter*, they are not used effectively. In some countries, for example, campylobacteriosis is notifiable, while in others the notification is food poisoning and *Campylobacter* is not always listed as a cause. Due to the scale of the problem, exhaustive surveillance is not feasible, but sentinel surveillance and cross-sectional studies are reasonable alternatives. As with other zoonotic foodborne diseases, campylobacteriosis bridges the concerns of both veterinary and human medicine and much of the progress to date are the result of collaboration between these disciplines. Food scientists concerned for public health have also joined the veterinarians and doctors. Nevertheless, collaboration is still uncommon in many parts of the world.

Needs

- All faecal samples cultured to test for enteric pathogens, such as *Salmonella* or *Shigella*, should also be tested for *Campylobacter*. This should be done for all

age groups. Clinical isolates of *Campylobacter* should also be tested for resistance to relevant antimicrobials.

- Laboratory data need to be linked with epidemiological data. Minimally, the denominator data should include specimen date, patient age and gender, the severity of the disease, hospitalisation, and travel history. This would help formulate intervention strategies at both local and national level.
- The detection and investigation of disease outbreaks should be the responsibility of both national and local surveillance programmes. Surveillance programmes should monitor the incidence and trends of the disease, as well as changes in disease patterns, such as changes in post-infection sequelae. Programmes such as ENTERNET may provide a useful model.
- Better education of the general public and public health officials as to the health risks posed by *Campylobacter*.
- Training programmes for food handlers, health care professionals and veterinarians should include public health issues directly related to *Campylobacter*.
- More cooperation between veterinarian, medical and food safety authorities and scientists is needed. Supporting interdisciplinary cooperation will increase the public health capacity for surveillance, outbreak response and disease control.

More cooperation between veterinarian, medical and food safety authorities and scientists is needed.

7.2 Developing countries

Current state

Enteric diseases are important causes of infant morbidity and mortality. It is estimated there are some 3 000 000 000 episodes of paediatric diarrhoea each year worldwide, which lead to an estimated 3 000 000 infant deaths annually. Among the pathogens causing paediatric diarrhoeal diseases, *Campylobacter* plays an important role and is particularly acute during weaning. Consequently, campylobacteriosis contributes significantly to malnutrition in infants and they represent an at-risk group. Immunocompromised patients are an additional at-risk group. This is particularly relevant given the AIDS epidemic sweeping the globe. The known sequelae due to campylobacteriosis, such as GBS, are poorly documented.

The disease burden of this pathogen in developing countries is not known because the laboratory infrastructure is inadequate and no surveillance networks exists. In particular, there is limited capacity for surveillance; outbreak response; and for the control of food and waterborne disease, all of which are basic public health skills. Building such capacity, and addressing the major sanitation and health concerns, will improve public health in those countries.

The disease burden of this pathogen in developing countries is not known because the laboratory infrastructure is inadequate.

Needs

- The burden of *Campylobacter* needs to be established more accurately in many countries. There is an urgent need to know what impact the illness has on public health and on the economy. This can only be addressed by enhancing the laboratory and epidemiological capabilities in these countries.
- Every paediatric faecal sample examined for enteric pathogens, such as *Salmonella* and *Shigella*, should also be examined for *Campylobacter*.

- Existing regional centres of excellence should be strengthened or new ones should be developed. National laboratories should be able to provide speciation, typing and antimicrobial susceptibility testing on *Campylobacter*. Regional centres could help by providing expertise and training to national laboratories.
- Laboratory-based surveillance networks should be established to facilitate the collection, analysis and communication of culture data to national and local communicable disease programmes.
- Education of the general public and public health officials as to the public health risk posed by *Campylobacter*.
- Training programmes for food handlers, health care professionals and veterinarians should also include public health issues related to *Campylobacter*. In particular, there is a need for specialist training in laboratory and surveillance methodologies, such as those used in the Global Salm-Surv approach.

7.3 Non human reservoirs of *Campylobacter*

Current state

Many non-human reservoirs of *Campylobacter* infection exist, including poultry, livestock, pets, water, milk and a wide range of foods, but their relative contributions to the disease burden is unknown. *Campylobacter* is also invariably present in faecal contamination. Accepted levels of *Campylobacter* in foods have not been defined and the impact of contamination levels on human disease is poorly understood. However, the risk of infection can be reduced by chlorinating water, pasteurizing milk, and by properly cooking and handling food of animal origin. There are few activities in place and they are part of ongoing control programmes.

There is a need to establish the relative contributions of different Campylobacter reservoirs to the burden of disease.

Needs

- Further research to establish which *Campylobacter* species and types are responsible for human disease, and to ascertain their reservoirs.
- To establish the relative contributions of different *Campylobacter* reservoirs to the burden of disease.
- To extend surveillance of antibiotic resistance among *Campylobacter* strains from food animals. Currently, this is carried out in only a few countries. The surveillance programmes should cover antibiotics that are clinically relevant to humans.
    - To harmonize surveillance methodologies, particularly those used to survey antibiotic resistance. Laboratory procedures should also be harmonized.
- To establish the relationship between *Campylobacter* in food and the health risk to the population. This requires appropriate population-based risk assessments to inform risk management.

8. Intervention and control of *Campylobacter*

Because *Campylobacter* is widely distributed in nature and in commercial food animal products, interventions can affect particular sources of infection without necessarily influencing human exposure. In Sweden, for example, *Campylobacter* infec-

tion has been reduced in poultry flocks, but the number of human campylobacteriosis cases has nevertheless increased, which has been attributed to foreign travel. Control measures must therefore be appropriate to individual sources and circumstances, and many factors, including the geographical region, culture and food availability, will dictate the types of control measures that can be applied. Control measures should be applied to all phases of food production, and education and training must be provided for personnel at all levels in the food production chain.

8.1 Developing countries

Current state
Campylobacter species are among a number of well-known enteric pathogens and they cause high levels of morbidity and mortality in infants and children. In humans, waterborne transmission is a major route of infection.

Needs

- Improve the hygiene of water supplies.
- Segregate human waste from human water supplies.
- Avoid human contact with animal waste.
- Keep animals away from children.
- Promote breast feeding.
- Pasteurize milk and follow proper hygienic measures for fermented dairy products. If pastuerization is impractical, milk for infants should be boiled.
- Treat diarrhoea by oral rehydration and use antibiotics only in severe cases.
- Follow WHO guidelines when implementing diarrhoeal disease control programmes.
- Improve hygiene practices in food handling

8.2 Developed countries

Current state
Poultry has been identified as the most important vehicle for *Campylobacter* transmission, but additional risk factors include other foods of animal origin, inadequately treated water, raw milk, contact with farm animals and pets, and foreign travel. There is only limited information on appropriate control measures.

Needs

- Improve the hygiene of water supplies.
- Action at the level of individual farms, and during food production and processing, to reduce *Campylobacter* transmission.
- More information about the epidemiology of *Campylobacter* species. A better understanding of *Campylobacter* sources and virulence characteristics, and of modes of transmission, would enable control measures to be better targeted.
- National agencies should take a leading role in educating and training all those involved in food preparation and handling.
- To develop a human vaccine for *Campylobacter*.

8.3 Specific control measures

Current state
The success of control measures varies between countries and locations. In Iceland, for example, lack of control in poultry and changes in consumption habits led to an epidemic in human campylobacteriosis from chicken. Subsequently, as *Campylobacter* levels in poultry were reduced, there was a corresponding reduction in human campylobacteriosis. In Scotland, the introduction of statutory milk pasteurization in the 1980s was associated with a dramatic reduction in the number of outbreaks of human enteric diseases, including campylobacteriosis. In 1996, the United States expanded changes in its slaughter sanitation procedures, based on a Hazard Analysis and Critical Control Point (HACCP) approach. Since then, *Campylobacter* infection rates have decreased 25% and *Campylobacter* contamination of poultry has also decreased. These examples also illustrate the different types of measures required to deal with this human health problem.

8.3.1 Poultry

Current state
A number of studies have found poultry to be the major source of human campylobacteriosis in the developed world, although little is known about how flocks become infected. Measures to control the causative agents focus on the farm. However, some intervention measures that are successful against Salmonella, such as competitive exclusion and vaccination, are less effective against *Campylobacter* species, or are unavailable.

Needs
- ¥ Further study into the use of antagonistic intestinal microflora to limit *Campylobacter* colonization.
- ¥ To investigate whether selected changes in poultry production reduce or prevent *Campylobacter* transmission. The pathogen is known to be relatively fragile and sensitive to changes in its environmental conditions. Its die-off may be encouraged by changing the litter conditions (e.g. acidification, desiccation), decontaminating the water supply and using resting periods between crops of birds.
- ¥ Further research into the development of a vaccine for poultry. So far this approach has not been productive, but further research should be stimulated.
- ¥ Consider paying a premium to farmers who produce poultry free of *Campylobacter*. This approached has been used in Sweden and Denmark, but may be a politically sensitive issue in some countries.

8.3.2 Biosecurity in poultry production

Current state
Data from research and surveillance programmes, as well as from commercial producers, show that the successful control of foodborne pathogens during poultry production depends on preventing or reducing the probability of flock colonization. This may only be feasible, however, if controlled environment housing is used, and this is not always the case. Some countries have used this approach for many years

to control *Salmonella*, but *Salmonella* is more easily controlled than *Campylobacter* and additional interventions may be required. At present, the types of additional interventions are ill defined, due to a lack of information about the sources and routes of transmission of *Campylobacter* species.

Needs

- More information about the effectiveness of biosecurity measures for poultry farms and about the impact the measures have on human campylobacteriosis.

8.3.3 Biosecurity for other food animals

Current state

Different control strategies are required for animals other than poultry, due to the absence of controlled environment housing and to differences in environmental exposure associated with production practices. For example, beef cattle will be exposed to *Campylobacter* while on pasture land or in feedlots, and studies on pigs have shown that *Campylobacter* is readily transmitted from sows to offspring. As a result, control measures are likely to be more difficult for these animals and novel approaches may be needed. In one farm set up with SPF sows, and using high levels of biosecurity, *Campylobacter* levels were reduced and the lower levels were maintained over time. This illustrates the potential value of an integrated approach to the control of *Campylobacter* in pigs.

Raw milk may also contain pathogenic bacteria even when produced under the highest levels of hygiene control, although pasteurized or otherwise adequately heat-treated dairy products are free from *Campylobacter*. Pets, too, may be a significant source of human campylobacteriosis in some countries and proper management of pet excrement and pet contact with children is needed.

Needs

- To optimizing slaughtering and butchering operations so that intestinal content is not transferred to meat surfaces, with due consideration to high-risk material such as offal.
- To discourage drinking raw milk, which can be a source of *Campylobacter*.
- The management practices for livestock manure and the treatment of abattoir wastewater should minimize the spread of *Campylobacter* from these sources.

8.3.4 Use of antibiotics in food animals

Current state

Throughout the world, there is a growing problem of antibiotic resistance in *Campylobacter*, particularly to fluoroquinolones. It is likely that this has largely arisen from the widespread use of antibiotics in agriculture.

There is a growing problem of antibiotic resistance in Campylobacter, particularly to fluoroquinolones.

Needs

- The use of antibiotics and other substances that are important in human medicine should be restricted in livestock as much as possible. The WHO guidelines on this topic should be followed.

8.3.5 Travel

Current state
Travel is perceived as a risk factor for *Campylobacter* infection. To reduce risk, recommendations developed by the WHO should be followed.

Needs

- ¥ Educate travellers about the risks involved in travelling and about how to avoid *Campylobacter* infections.

8.3.6 Food preparation and handling

Current state
Cross contamination during food preparation contributes to the spread of *Campylobacter*. In particular, barbequed poultry has been associated with transmission. Because *Campylobacter* cannot multiply outside the human intestinal tract, exposure is limited to the levels found in food. Appropriate precautions in handling and preparing foods of animal origin will reduce cross contamination and food handlers should refer to the WHO guidelines.

Needs

- ¥ Better education and training of all involved in food preparation and handling.
- ¥ To evaluate freezing as a method for reducing product contamination. Freezing reduces the level of *Campylobacter* contamination by approximately 100-fold and it may be possible to exploit this to reduce product contamination.
- ¥ Encourage appropriate standards of personal hygiene among farmers, abattoir workers and food handlers. Workers are particularly prone to *Campylobacter* infection when first employed in these occupations.
- ¥ Regulators and producers should evaluate the producer s obligation to minimize *Campylobacter* contamination of its products.
- ¥ Future regulations pertaining to the levels of *Campylobacter* on foods should be based on scientific evidence that measures the relationship between exposure and disease. This could be accomplished with a quantitative risk assessment. Presently, the public health significance of different levels of *Campylobacter* contamination is unknown.

8.3.7 Processing

Current State
It is difficult to control *Campylobacter* during poultry processing, because of the high incidence of this pathogen in poultry flocks and the high levels in chicken intestines. Although processing itself does not solve the problems, applying HACCP principles can optimize hygiene control. Experience has shown that certain modifications of the processing operation can produce small but significant reductions in contamination of carcasses with *Campylobacter* species. Further reductions will require more fundamental changes in the process that is currently used.

Needs

- More hygienic processing equipment. This may involve new design and use concepts in processing itself.
- A more effective method for decontaminating carcasses that would be suitable for the highest rates of carcass production. For example, since *Campylobacter* is relatively sensitive to drying, it may be possible to exploit the drying effect of cold air to reduce carcass contamination.
- Whenever possible, giblet packs should not be included with packaged carcasses. Edible offal, including hearts, livers, necks and gizzards can be heavily contaminated with *Campylobacter*.
- To improve the cleaning of the crates used to transport birds from the farm to the processing plant. Often, the crates are inadequately cleaned and disinfected between uses and can transmit *Campylobacter* to an uninfected flock during transportation.
- To investigate the possibility of using modified atmosphere packaging technology to enhance *Campylobacter* die-off. More information is needed about the survival of *Campylobacter* species when stored in modified atmospheres.
- When the prevalence of *Campylobacter* infection in poultry flocks is sufficiently low, it is recommended that positive flocks be processed last in the day, to avoid the risk of flock-to-flock contamination.

8.3.8 End-product decontamination by irradiation

Current state

Although scientific evidence suggests that using ionizing radiation to decontaminate foods is both safe and effective, there is considerable resistance among consumers to its use. *Campylobacter* is relatively sensitive to low-dose radiation treatment and could be readily eliminated from poultry meat products by this means, without any adverse effects on product quality. The use of irradiation technology should be considered when attempting to control *Campylobacter* in the poultry industry. With or without irradiation, the primary level of control should be on the farm. Irradiation should never be used as a substitute for hygienic measures.

Needs

- More attention should be given to educating consumers on the merits of food irradiation, and to developing appropriate strategies for test marketing irradiated poultry.
- To consider the economic constraints of installing radiation facilities, and the risk of prejudicing conventional control measures at all stages of poultry production if irradiation were to be introduced.

Table 1. Current recommendations for the collection and transport of samples that should be investigated for the occurrence of *Campylobacter*

	Enteric infections	Bacteremia	Food/waterborne	Animal infections
Type of sample	Stool, rectal swab	Blood	Refer to standards	Stool, rectal swabs
Sample transport	Refrigeration or suitable transport	Commercial blood culture bottles	Refer to standards	Refrigeration or suitable transport
Medium	Cary Blair or Amies			Cary Blair or Amies

Table 2. Current recommendations for the culture of thermophilic *Campylobacter*

Pathogen	Culture medium	Incubation atmosphere	Incubation temperature	Incubation time
C. jejuni, C. coli	MCCDA enrichment [a]	Microaerobic	42ßC or 37ßC	2-4 days
Campylobacter species other than C jejuni/coli [d]	Filtration [b]	Microaerobic plus 6% H_2 [c]	37ßC	Up to 6 days

[a] Enrichment cultures may be useful for looking for low numbers of organisms. This situation can arise during surveillance, for example, or when screening for asymptomatic carriers and postinfectious sequelae.

[b] The faecal suspension is applied to a 0.65 (m filter. The growth medium (e.g. tryptose soy, Mueller-Hinton, Columbia agars) must contain at least 5% sheep blood (or equivalent, such as horse blood) and should not contain any antibiotic supplements.

[c] Isolation of *Campylobacter* species other than *C. jejuni* and *C. coli* needs a microaerobic environment enriched with hydrogen. The optimal concentration of hydrogen has not yet been established, but H2 concentrations >10% may form explosive mixtures and caution should be exercised.

[d] *Campylobacter* species can be identified by colony morphology, Gram stain and oxidase reactions. *C. jejuni* is most economically identified with the hippurate hydrolysis test. For non-*C. jejuni* species, other phenotypic and/or genotypic assays should be used.

Annex 1. Programme

Tuesday, 21 November 2000

1230 - 1300 **Arrival and registration**

Opening session

1300 - 1330 Welcome to the Danish Veterinary Laboratory
Dr Knud B¿rge Pedersen, Director DVL

Welcome and introduction to the WHO Campylobacter Consultation
Dr Klaus St hr, WHO

Practical information about the meeting (election of chairman, vice chair, rapporteur)
Dr Klaus St hr, WHO

Campylobacteriosis - Disease aspects, incidence and trends in different countries and burden on public health

1330 - 1355 Campylobacteriosis in humans - A historical overview
Professor Jean-Paul Butzler

1355 - 1430 Incidence, trends and sources of campylobacteriosis in developed countries
Dr Robert V. Tauxe

1430 - 1450 Break

1450 - 1525 Incidence, trends and sources of campylobacteriosis in developing countries
Prof. Akitoye Coker

1525 - 1555 Health burden due to infections with thermophilic *Campylobacter* spp.
Dr. Arie Havelaar

Epidemiology of *Campylobacter* infections in humans (sources of infection and routes of Transmission)

1555 - 1625 What have we learned about *C. coli*/jejuni from bacteriological typing studies?
Dr Stephen W. On

1625 - 1655 What have we learned about *C. coli*/jejuni from anlytical epidemiological studies?
Dr Kaare M¿lbak

1655 - 1705 Break

1705 - 1750 General Discussion

1750 - 1800 Information about Campylobacter risk assessment activities in WHO
Dr. Allan Hogue

1800 - Reception buffet dinner

Wednesday, 22 November 2000

Epidemiology of *Campylobacte*r infections in humans (continued)

0900 - 0930 Epidemiology of other Campylobacter species
Dr Albert Lastovica

0930 - 1000 Major risk factors from human campylobacteriosis - Overview
Dr Robert V. Tauxe

1000 - 1020 Break

1020 - 1050 Emergence of antimicrobial resistance in Campylobacter: The consequences
for incidence, clinical course, epidemiology and control
Dr Heriberto Fernandez

1050 - 1120 What can be learned from surveillance and register studies
Dr Kaare M¿lbak

1120 - 1150 Combining typing data and epidemiological information in Campylobacter surveillance - new opportunities
Dr. Jenny Frost

1150 - 1230 General discussion

1230 - 1330 Lunch

Isolation and identification methods

1330 - 1420 Methods for isolation of Campylobacter from human clinical, animal, food
and water specimens
Dr. Frederick James Bolton

1420 - 1530 Break

1530 - 1600 Methods for identification of Campylobacter
Dr Peter Vandamme

1600 - 1730 General discussion (what are the major information needs, where are the knowledge gaps and how do we fill these gaps?)

1900 - Conference dinner

Thursday 23 November 2000

Antimicrobial resistance, control and surveillance programmes

0900 - 0930 Strengths and weaknesses of bacterial typing tools for the study of Campylobacteriosis epidemiology (Serotyping, PFGE typing, Ribotyping, AFLP typing, PCR-RFLP, RAPD)
Dr Dianne G. Newell

0930 - 1010 Strategies for pre-harvest control of Campylobacter in chickens, swine and Other food animals: A review
Dr Norman J. Stern

1010 - 1030 Break

1030 - 1110 Strategies for post-harvest control of Campylobacter: A Review
Dr Geoffrey Mead

1110 - 1200 General discussion (why so few programmes and so little progress?)

1200 - 1300 Lunch

1300 - 1330 Surveillance programmes: Their function as a tool for control of Campylobacteriosis including Introduction to WHO Global Salmonella- Surveillance as a model of an international surveillance network
Dr Henrik C. Wegener

Working groups

1330 - 1730 *Surveillance:* Current state and needs for strengthening of national laboratory based surveillance programmes
Chairman: Dr Dianne G. Newell
Working group rapporteur: Dr Henrik C. Wegener

Laboratory methods: State of the science, knowledge gaps, identify needs for harmonisation, research and development
Chairman: Dr Stephen Won.
Working group rapporteur: Dr Jorgen Engberg

Epidemiology: State of the science, knowledge gaps, identify surveillance and research needs
Chairman: Dr Robert V. Tauxe
Working group rapporteur: Dr Eva Moller Nielsen

Control and intervention: State of the science, knowledge gaps, identify research needs
Chairman: Dr Norman J. Stern
Working group rapporteur: Dr Mogens Madsen

Friday 24 November 2000

0900 - 1200	Working groups continue
1230 - 1330	Lunch
1330 - 1600	Working groups Working groups continue
1600 - 1730	Working group presents first draft
1730 -	Working group prepares final draft

Saturday 25 November 2000

Plenary

0900 - 1020	Working groups present draft report
1020 - 1040	Break
1040 - 1200	Working groups present draft report
1200 - 1230	Discussion and acceptance of final document: Preparation of Recommendations
1230 - 1300	Closing address. *Dr Klaus St hr, WHO*

Annex 2. List of Participants

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Annex 3. WORKING PAPERS

As presented at the WHO expert consultation. The content of the working papers is solely the responsibility of the authors

Contents

***Presentations*:**

1. Campylobacteriosis in Humans - A Historical Overview
Professor Jean-Paul Butzler *4*

2, Incidence, Trends and Sources of Campylobacteriosis in developed countries: An overview
Dr. Robert V. Tauxe *8*

3. Incidence, Trends and Sources of Campylobacteriosis in developing countries: An overview
Prof. Akitoye O. Coker *10*

4. Health burden due to infections with thermophilic *Campylobacter* spp.
Dr. Arie Havelaar *15*

5. What have we learned about *Campylobacter coli/jejuni* from bacteriological typing studies?
Dr. Stephen W. On *19*

6. What have we learned about *Campylobacter coli/jejuni* from anlytical epidemiological studies?
Dr. Kåre Mølbak *24*

7. International risk assessment of microbiological hazards in foods
Dr. Allan Hogue *25*

8. Epidemiology of other *Campylobacter* species
Dr. Albert Lastovica *27*

9. Major risk factors for Human Campylobacteriosis – An Overview
Dr. Robert V. Tauxe *31*

10. Emergence of antimicrobial resistance in *Campylobacter*: The consequences For incidence, clinical course, epidemiology and control
Dr. Heriberto Fernandez *33*

11. What can be learned from surveillance and register studies
Dr. Kåre Mølbak *39*

12. Combining typing data and epidemiological information in *Campylobacter* surveillance – new opportunities
Dr. Jenny Frost *47*

13. Methods for isolation of *Campylobacter* from humans, animals, food and water
Dr. Frederick James Bolton *53*

14. Methods for identification of *Campylobacter*
Dr. Peter Vandamme *60*

15. Strengths and weaknesses of bacterial typing tools for the study of Campylobacteriosis epidemiology
Dr. Dianne G. Newell *67*

16. Strategies for controlling *Campylobacter* spp. in poultry
Dr. Norman J. Stern *72*

17. Strategies for post-harvest control of *Campylobacter*: A Review
Dr. Geoffrey Mead *76*

1. Campylobacteriosis in humans (A historical overview)

Jean-Paul Butzler

Although *Campylobacter spp.* were not recognized to be human pathogens until the 1970's, they probably have caused human illness for centuries.

In 1886 Theodor Escherich published a series of articles in the Münchener Medizinische Wochenschrift. He described spiral bacteria which he found associated with large intestinal mucus in 16 of 17 children who had died from diarrhoeal disease. Growth on solid medium was unsuccessful. Furthermore, he observed spiral bacteria microscopically in stool specimens of 35 of 72 infants suffering form enteric disease. But despite the increased frequency observed, he thought the spiral bacteria played no etiological role (8). Unfortunately, these articles published in German went unrecognized for many decades. Manfred Kist reported Escherich's findings at the Third International *Campylobacter* Workshop held in Ottawa in 1985 (12). An event that took place in Illinois in May 1938 is now regarded as the first well documented instance of human *Campylobacter* infection (13). It was a model investigation of a milk borne outbreak of diarrhoea that affected 355 inmates of two adjacent state institutions. Fecal cultures from the 73 victims tested were negative (microscopy was positive in 31) but organisms resembling " V. *jejuni*" were grown in broth cultures of the blood of 13 victims. Vibrio fetus was first found in man in 1947 by Vinzent who isolated it from the blood of three pregnant women admitted because of fever of unknown origin (24). The illness lasted about four weeks and two of the three women aborted. On examination, the placenta revealed large necrotic and inflammatory areas. The third patient was treated with penicillin and sulphonamids; She had a full-term baby, whose birth weight was 2.130 g. The agent isolated from the blood cultures was identified as V. fetus intestinalis and the authors therefore regarded *C. fetus* as a new causative agent of abortion. In humans *C. fetus* is rarely isolated from stools. The pathology caused by V. fetus differs from that caused by V. *jejuni*.

V. fetus nearly always attacks debilitated individuals. The clinical data from the described cases of *C. fetus* infection argue for a non-specific pathology. All the syndromes described are a result of bacteraemia. In 1957 E. King described a vibrio as a causative agent of *enteritis* in infants and young children (10). It could be distinguished from V. fetus by its biochemical and antigenic properties. She called it related Vibrio. This condition was, for a long while unrecognized. Indeed until 1972, only 12 cases of *C. jejuni* infections were known : 7 infants, 2 children and 3 adults. Of seven infants and two children, only one infant had any pre-existing pathology. The three adults were debilitated and died. On post-mortem examination lesions of hemorrhagic necrosis were found in one patient in the jejunum and in the middle part of the ileum. There were no lesions in the colon. In all these cases of *enteritis*, blood cultures yielded *Campylobacter*. The authors did not succeed in isolating the agent from the stools. Blood culture is an uncommon diagnostic procedure in *enteritis*. Moreover, blood culture is not a common procedure in infants and young children. This explains why disease due to *C. jejuni* was, for a long while, unrecognized in man. King described the close similarity of the biochemical characteristics of *C. jejuni* and of *C. Coli*. An epidemiological link was found in an adult farmer, who had been in daily contact with poultry. King showed that the *C. jejuni* isolated from the patient had the same biochemical and serological characteristics as the *C. Coli* isolated from the poultry (11). The optimal growth of the organism lies at 42°C. This might suggest an adaptation to warm-blooded animals such as poultry. E. King's vision paved the way. Sadly, she died of cancer in 1966 and so she never saw how her "related vibrios" ended up heading the list of enteric pathogens. The crucial step – the isolation of *Campylobacter* from feces – was accomplis-

hed in 1968 by Dekeyser at the National Institute for Veterinary Research, Brussels, Belgium in conjunction with Butzler and his team at the St Pierre University Hospital and published in 1972 (6). A 20 year-old female was admitted on July 18, 1968 to the St Pierre University hospital in Brussels with severe diarrhoea and fever (40°C). She had no underlying pathology. A related vibrio (*C. jejuni*) was isolated from the blood and after use of a special filtration technique also from the feces. This technique consisted of differential filtration of fecal suspensions through 0.65 µm pore size filters which allowed *Campylobacter* organisms to pass through. The filtrate was then inoculated onto a selective medium. No other enteric pathogens were isolated in the stools of this patient. This first faeces culture demonstrated intestinal infection as the origin of the bacteraemia. This clinical case was the starting point of a strong collaboration between Butzler and Dekeyser which included a search for *Campylobacters* in the stools of healthy individuals and patients with diarrhoea; a search for specific serum antibodies in these groups and the collection of clinical data and the elaboration of a therapeutic scheme. *C. jejuni* was isolated from 5.3% of 3.800 diarrhoeic stools and only from 1.6% of 7.200 normal stools (1). Specific complement fixing antibodies to the strain of *C. jejuni* isolated from their stools were demonstrated in children with diarrhoea (3).

Most important was the finding of the high susceptibility of *C. jejuni* for erythromycin (4). Once we knew how sensitive related vibrio (*C. jejuni*) was to erythromycin, we used this antibiotic as a therapeutic test (3).

The cessation of the diarrhoea together with the disappearance of related vibrio (*C. jejuni*) from the stools could be used as an argument in favour of *C. jejuni* being the cause since erythromycin has no effect on the ordinary intestinal pathogens. In all the treated cases, erythromycin caused the symptoms to disappear rapidly. Several cases of diarrhoea due to related vibrio (*C. jejuni*) were resistant to colistin treatment and the stools cultures remained positive. They subsequently responded readily to erythromycin (3). The invasive power of related vibrio (*C. jejuni*) was demonstrated in poultry (3). Finally antigenic typing of the isolated related vibrio (*C. jejuni*) strains was performed by agglutination and complement fixation tests using antisera raised from reference strains of vibrios (*Campylobacter*). Close antigenic relationship and even identity was shown between human strains and strains isolated from poultry, sheep and pigs (3). Butzler's and Dekeyser's papers although published in international journals elicited no response until they were picked up by Skirrow in the United Kingdom in 1976 (20). Skirrow's first encounter with a *Campylobacter* was with an organism grown from the blood of a 1 month old baby with febrile diarrhoea. This was an organism that really caught the imagination. A search of the literature turned up Butzler's papers which were viewed with surprise, and not a little scepticism in view of the absence of any subsequent papers on the subject ! However their potential importance demanded that they should be put to the test. Within days, feces were being filtered onto blood agar and isolations were forthcoming from samples from patients with diarrhoea. The filtration technique for culturing *Campylobacters* proved too time-consuming for the routine investigation of large numbers of specimens. Skirrow in 1977 published a more simple technique for culturing *C. jejuni* and *C. Coli* from stool specimens, which allowed widespread isolation of these organisms (20). During the 1980s, major advances included the development of improved isolation techniques. Following this progress it became obvious that the incidence of *C. jejuni* usually exceeds that of better-known human enteric pathogens such as *Salmonella spp*, and that the economic effects of *Campylobacter* infections are considerable.

That *Campylobacters* were not restricted to the developed world became clear as isolations from Rwanda (7) confirmed an early report from Zaire by Butzler (2). The year 1979 saw the publication of the first full account of *Campylobacter enteritis* in humans (5). Later pioneering studies of John Penner (15) and Hermy Lior (14) gave us serotyping schemes of great value. These complimentary schemes, aided by biotyping and phage typing, still form the basis of strain typing but major advances are being made in genotyping methods.

1. Taxonomy

Campylobacter fetus and *C. jejuni* previously belonged to the Vibrio genus because of their comma-like morphology in young cultures. They are distinguished from V. cholerae and V. parahemolyticus and other species by their very different biochemical and antigenic characteristics and the guanosine-cytosine content of their DNA. For this reason Sebald and Véron in 1963 (19) classified them in a different genus *Campylobacter* (Greek : "campylo": curved and "bacter" : a rod). The clarity afforded by genetic analysis, not only enabled new species to be neatly categorized but also caused some former *Campylobacters* to be transferred to Helicobacter and others to be given a new genus, Arcobacter (22, 23).

2. A new clinical problem

Although the majority of cases of Campylobacteriosis are characterized by a self-

limited diarrhoeal illness, serious sequelae can occur. In 1982 Rhodes et al published the case of a patient who developed Guillain-Barré syndrome (GBS) 2 weeks after the onset of *Campylobacter enteritis* (18). The first survey of GBS and *Campylobacter* infection, a retrospective one, was carried out in Australia (9). This study and subsequent prospective studies in the United Kingdom and Japan confirmed the association. It is now clear that *Campylobacter enteritis* is the most frequently identified antecedent event in GBS (16,17).

3. Some important things we learned about *Campylobacter enteritis* all over the years

3.1 *Campylobacter enteritis* is the most frequent form of acute bacterial diarrhea in developed countries. It affects people of all ages and is prominent in young adults, especially young men.

3.2 In developing countries the disease is confined to young children who develop immunity early in life trough repeated exposure to infection.

3.3 The symptoms of *C. jejuni* infection are usually mild but systemic and post infectious manifestations such as the Guillain-Barré syndrome may occur.

3.4 In general, *Campylobacter enteritis* is a self-limiting disease and the isolation of the organism from the stools does not warrant chemotherapy. In the absence of chemotherapy the feces remain positive for about 2 to 7 weeks.

3.5 Antimicrobial treatment is indicated in prolonged disease with severe symptoms, in high fevers or with bloody stools, in relapses, in pregnancy and in immunocompromised patients.

3.6 In industrialized countries macrolides continue to be the drugs of choice for the treatment of *Campylobacter* infections because the prevalence of erythromycin resistant strains remains low and stable. The situation for quinolones is totally different. Several authors have shown a remarkable increase in the level of resistance since the introduction of norfloxacin and ciprofloxacin and the inclusion of enrofloxacin in veterinary practices.

3.7 The infection is seasonal in temperate climates. About twice as many infections occur in summer than winter.

3.8 The infection is a zoonosis. The reservoir of infection is in wild and domestic animals, particularly birds. Chickens constitute by far the largest potential source of human infection.

3.9 Transmission is mainly indirect via food, milk and water. Direct transmission is mainly occupational (farmers, butchers, abattoir workers, poultry processors), but pets can bring infection into ordinary homes. In developing countries exposure to the feces of *C. jejuni* infected live chickens in the household is the predominant factor for childhood *C. jejuni* diarrhea.

3.10 Prevention depends upon the purification of all water supplies, the heat treatment of all milk sold for human consumption, the hygienic handling of all raw meats, especially poultry, in kitchens, and the control of infection at all stages of poultry production. In developing countries penning chickens outside the home and preventing contact with their feces substantially reduces transmission of *C. jejuni*

3.11 The problem of *Campylobacter enteritis* remains undiminished. Control of infection in chickens and better information of the public how to handle food correctly are crucial steps for prevention (21).

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2. Incidence, trends and sources of Campylobacteriosis in developed countries: An overview

Robert V. Tauxe

An extended abstract of presentation

Campylobacter is the most commonly isolated bacterial enteric pathogen in the United States and many other developed countries. This infection represents a substantial burden to public health. In the United States, the incidence of diagnosed clinical infections due to *C. jejuni* or *C. Coli* identified through active laboratory-based surveillance was 25.2 per 100,000 population in 1997. These diagnosed and reported infections represent a small fraction of the total number of actual infections, because many who are ill do not visit a physician, and many medical consultations do not result in a stool culture. Accounting for these effects, we have estimated that in the case of *Salmonella* there are 38 infections for every reported case. Applying this multiplier to *Campylobacter*, we estimate there are 2,400,000 infections, nearly 1% of the US population per year. These infections result in an estimated 13,000 hospitalizations, and 124 deaths each year. Incidence of reported infections in Northern European countries has been from 60 to 90 cases per 100,000; these may represent only 1-10% of the actual number of infections. Post infectious sequelae are part of the burden. In the United States, if we estimate that 1/1000 of the infections lead to Guillain Barre syndrome (GBS), this would represent approximately 2,400 GBS cases per year.

In the last 20 years, *Campylobacter* infection rates have risen substantially in many developed countries. Part of this increase may be due to improvements in detection and reporting, but part is likely to reflect a true increase in infections. For example, the incidence of reported infection in New Zealand increased from 14 to 120 per 100,000 between 1981 and 1990, and to an extraordinary 363 per 100,000 by 1998. In Denmark, rates remained relatively constant from 1980 to 1990, but increased nearly threefold from 1990 to 1998. In the United States, the incidence has actually declined from the peak in 1997 of 25.2 to 17.3 per 100,000 in 1999. This decline is not explained by changes in culturing or reporting practices, and may represent a true reduction in risk. This decline has occurred at the same time as substantial improvement in the disinfection of water used in chill tanks in the poultry industry, and other slaughter sanitation improvements. In the U.S., we have noted substantial variation by region; ranging from 6 per 100,000 in Maryland to 32 per 100,000 in the area of San Francisco, California. These are likewise not explained by differences in culturing or reporting practices, and may represent real regional differences in risk.

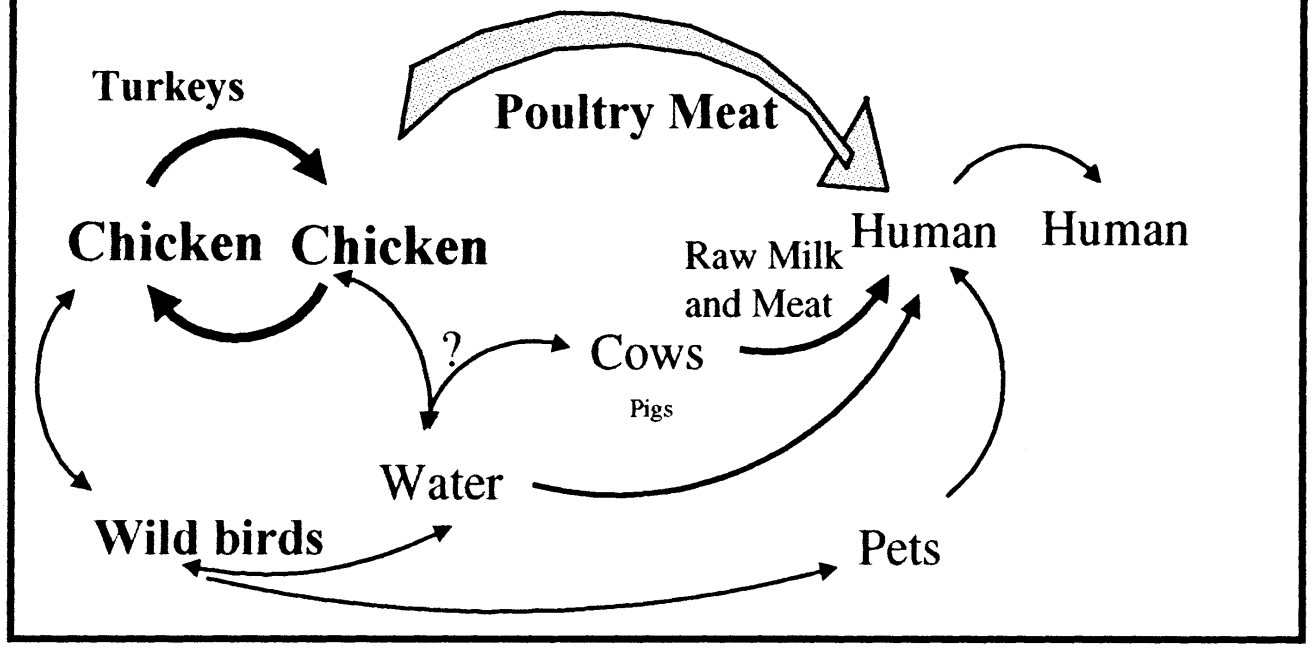
The seasonality of *Campylobacter* infections in the developed world generally exhibits a well-defined summer peak, that is more pronounced with increasing latitude. The distribution by age and sex is also similar in most countries. Campylobacteriosis affects all ages, but has a distinctive bimodal distribution, affecting particularly children younger that 4 years, and young adults between 15 and 44 years. The incidence in males is 1.2 to 1.5 times higher than in females, and the male predominance is particularly evident in the young adult peak. Persons with acquired immunodeficiency syndrome (AIDS) are at higher risk of acquiring *Campylobacter* infections, and persons with extreme immunodeficiency appear to be at higher risk for invasive disease, should they become infected.

One public health challenge of Campylobacteriosis is that many infections are acquired during international travel, typically to other parts of the developed world. Such exposure may account for 5-10% of cases in the United States, 10-15% of cases in Great Britain and Denmark, and 50-65% of cases in Sweden and Norway. Travel in other countries is a complex exposure that includes a variety of exposures.

Fluoroquinolones, such as ciprofloxacin, are commonly used for the treatment of infections caused by *Campylobacter*. Fluoroquinolone (FQ) resistance has been reported since the end of the 1980's from Europe and Asia, and more recently, since 1995 in the United States. The prevalence of FQ-resistant *Campylobacter jejuni* in the United States was 0% in 1990, and increased to 13% in 1997 to 18% in 1999, following the approval of FQ use in poultry in 1995. In contrast, in Australia, where FQs are not used in poultry, human isolates of *Campylobacter* remain susceptible to fluoroquinolones.

The contribution of other *Campylobacter* species to the overall public health burden remains unmeasured. They are rarely identified by standard methods, typically represent less than 1% of reported *Campylobacter* infections reported from clinical laboratories, and most often are detected as opportunistic pathogens in an immunocompromised host. This was the status of *C. jejuni*, before selective diagnostic media were developed and widely used. In the future, better diagnostic methods may increase the perceived public health burden of other *Campylobacters*.

Figure 1. General schema of *Campylobacter jejuni* transmission in the developed world



3. Incidence, trends and sources of Campylobacteriosis in developing countries – An overview

Akitoye Olusegun Coker

1. Introduction

Campylobacteriosis is a collective description for infectious diseases caused by members of the bacterial genus, *Campylobacter*. The only form of Campylobacteriosis of major public health importance is *Campylobacter enteritis* due to *Campylobacter jejuni* and *C. Coli*. The rate of *Campylobacter* infections has been increasing, with the number of cases often exceeding those of *Salmonella* and Shigella. This increase as well as the expanding spectrum of diseases caused by the organisms has necessitated the need to improve the understanding of the epidemiology and control of Campylobacteriosis.

Priorities for surveillance and control of infections of public health importance in developing countries have focused on diseases such as malaria, tuberculosis, trypanosomiasis, onchocercasis and schistosomiasis. There are also programmes for diarrhoea and acute respiratory infections. These programmes have extensive support from the World Health Organisation (WHO).

Campylobacter is one of the most frequently isolated bacteria from stools of infants with diarrhoea in developing countries resulting from contamination of food or water. However, there are generally no national surveillance programmes for Campylobacteriosis in most developing countries despite the burden of disease associated with the infection. Most of the initial data available on Campylobacteriosis in developing countries were as a result of support provided by the WHO to many laboratories in the developing countries including grants for epidemiologic studies and Lior serotyping antisera provided by the Public Health Service of Canada.

In the next sections an overview of the incidence, trends and sources of human Campylobacteriosis in developing countries is presented taking into cognisance the World Health Organisation Regions.

2. Incidence

Generally in developing countries there are no national surveillance programmes for Campylobacteriosis to have incidence values in terms of number of cases for a population. Estimates of the incidence of infection are from laboratory-based surveillance of pathogens responsible for diarrhoea. *Campylobacter* isolation rates range from 5 to 20%. Table 1 shows the values for countries in WHO regions from studies on children under-five-years. The incidence of *Campylobacter enteritis* in developing countries is estimated at 0.4 episodes per child year, or 40,000/100,000 for children younger than 5 years. The incidence in the general population may be much higher than 90/100,000. The isolation rate in most developing countries has increased since the initial reports. For example, isolation rate in Lagos, Nigeria increased from 5.2% in 1984 to 16.5% in 1994.

3. Trends

3.1 Age

In studies from Latin America and Africa, *Campylobacter* is the most commonly isolated bacterial pathogen from children with diarrhoea under 2 years. In Central African Republic, *Campylobacters* were statistically associated with diarrhoea only before the age of 6 months. In Nigeria all the isolates of *Campylobacter jejuni* are still from children under two years. In Tanzania, *Campylobacter* infections are more important in children younger than 18 months, than in older ones. The excretion rate in Liberian children

increased with age. In Chengdu, China, isolation rate of *C. jejuni* in diarrhoeal children was rising with increasing age up to 12 months of age, tending to stabilize with increasing age after 12-24 months of age. In Thailand, *Campylobacter* species were associated with 18.8% of cases among children younger than 12 months, 12.3% of cases among those aged 12 to 23 months, and 10.3% of cases among those aged 24 to 59 months. In Mexico City the highest isolation rate was in the 7- to 12-month age group. In Algeria no statistically significant difference was found after stratification by age while in Bangladesh, the infection rate was significantly higher ($P<0.05$) amongst children up to 1 year of age (32.8%) compared to those aged over 1 year (15.9%). In Egypt, children less than 1 year of age were at greatest risk of *Campylobacter* infection with 32.6% of diarrhoeic patients culture positive, compared to 14.3% of controls.

3.2 Polymicrobial infections involving *Campylobacter*

In developing countries *Campylobacter* is isolated relatively frequently with another enteric pathogen in patients with diarrhoea. In a study in which a cohort of 111 children from Bangui, Central African Republic, was followed for enteric *Campylobacter* infection from birth until the age of 2 years *Campylobacters* were isolated from 41 (11.7%) of the 349 episodes, but in half of them another enteric pathogen was also isolated. *Campylobacter* species were isolated from 18% of Thai children with diarrhoea, and another enteric pathogen was isolated from half of the patients in whom *Campylobacter* was isolated. Pure *C. jejuni* culture was obtained in 18 of 35 samples from 385 hospitalised patients with acute *Campylobacter enteritis* in Bombay, India; the other 17 samples showed polymicrobial infection or infestation. In Egypt, patients with *Campylobacter*-positive diarrhoeal stools were frequently co-infected with rotavirus (28.6%) or Giardia lamblia (24.5%).

3.3 Isolation of *Campylobacters* in healthy children

In developing countries recovery of *Campylobacters* from children without diarrhoea is common. In a study in South Africa after the age of 9 months, there was no difference in the isolation rate of *Campylobacter* species between children with diarrhoea and well children. It appears that enteric infections with *Campylobacters* among children in Calcutta, India are common but often asymptomatic. The difference between the isolation rates of *Campylobacters* in those cases in which no other enteric pathogen was found (4.8%) and controls (6.2%) was not significant ($P> 0.05$). Furthermore, in a healthy rural population in rural India *Campylobacter* was the bacterial intestinal pathogen most frequently isolated from their stool samples. In Algeria, *Campylobacters* were isolated in 14.9% of the 247 healthy children. *Campylobacter jejuni* was not found in a significantly higher percentage of diarrhoea than in control children in a hospital-based study in Northern Thailand. It was isolated from the stools of 14 of 208 diarrhoea (6.7%) and 6 of 108 (5.5%) control patients. In Nigeria, *Campylobacter* was recovered from 2% of control stools examined in a study in Ile-Ife whereas no isolates were recovered from control patients in Lagos. *Campylobacter* species was more prevalent among control infants from a community in Guinea-Bissau.

3.4 Seasonal variation

Campylobacter isolation peaks in Mexico, Nigeria, Peru, Thailand and India (Varanasi) was observed in dry season with rainy season peaks in Central African Republic, India (Calcutta) and Egypt. In a study in the coastal area of Kenya and in a family cohort population in rural Egypt, no significant seasonal variation was observed. Epidemics are not reported in developing countries to facilitate an exact seasonal variation. However, it has been concluded from studies that there is a lack of seasonality due to lack of extreme temperature variations in tropical climates, and the apparent lack of epidemics might be explained by poor surveillance.

3.5 Distribution of *Campylobacter* species

Campylobacter jejuni and *C. Coli* remain the two main species isolated from most studies in the developing countries. It has been proposed that other species may be of importance but lack of diagnostic capacities has prevented the actual distribution. The isolation rate of *C. jejuni* exceeds that of *C. Coli* . Using Lior's biotyping and serotyping methods, *Campylobacter jejuni*/coli strains have been classified in various studies. The distribution of strains isolated in Central African Republic are 31.1% *C. jejuni* I, 11% *C. jejuni* II, 2.4% *C. jejuni* III, 44% *C. Coli* I and 11.5% *C. Coli* II. No significant difference was observed in the distribution of biotypes or serogroups between strains from healthy and diarrhoeic children in Central African Republic and Tanzania. Out of 101 *Campylobacter* isolates from Nigerian children with or without gastroenteritis, 53 (52.5%) were *C. jejuni* biotype I, 29 (28.7%) were *C. jejuni* biotype II, 10 (9.9%) were *C. Coli* biotype I, and 9 (8.9%) were *C. Coli* biotype II. Serogroups 1, 8, 11, 20, 28, 29 and 45 occurred in ill children and are probably more virulent strains. In Chile, analysis of isolates from human, animals and drinking water show that biotypes I and II accounted for 96% of *C. jejuni* isolates, the other 4% being biotype IV but the two biotypes of *C. Coli* were about equally

represented. A total of 28 serogroups (Lior's heat-labile antigens) were identified. Lior 13, 9, 79, 2 and 4 were prevalent among the *C. jejuni*, while Lior 8, 21 and 29/75 were prevalent among the *C. Coli* isolates. These serogroups accounted for 73% all isolates. The distribution of biotypes and serogroups in patients and asymptomatic persons were similar. In Saudi Arabia, 69% were *Campylobacter jejuni* (mostly biotype IV) and 31% *C. Coli*. Serogroups 5 and 23 (Penner scheme) and phage type 125 (Preston scheme) were most frequently isolated. In Bangladesh, of 102 isolates from man, 74% were typable and serotypes 53, 15 and 22 predominated. Of 26 isolates from animals, 65% were typable and serotypes 15 and 53 occurred frequently. The diarrhoeal illnesses associated with different serotypes were similar. *C. jejuni* biotype I was more frequent among Tunisian strains. Isolates from Cape Town, South Africa were 95.4% *C. jejuni* biotype I, 1.5% *C. jejuni* biotype II and 3.1% *C. Coli*. The most common serotypes in order, were: 4, 2, 12, 23/36 and 19. In Calcutta, India, *C. Coli* serogroup LIO 46 biotype II was the most frequently encountered strains (14.5%), followed by *C. Coli* serogroup LIO 29, 55 biotype II (10.5%) and *C. jejuni* serogroup LIO 54, biotype I (5.5%).

3.6 Antibiotic Resistance among *Campylobacter* isolates

There is an increasing rate of resistance to antibiotics used for treating *Campylobacter* infections is developing countries. Erythromycin is drug of choice for Campylobacteriosis but resistant strains are common among strains in Bangkok, Thailand. A significant number of the isolates (14.8%) from children in an urban community in Harare, Zimbabwe showed multidrug resistance to erythromycin, tetracycline and gentamicin. In 1984, 82% of *Campylobacter* strains from Lagos, Nigeria were sensitive to erythromycin but ten years later only 20.8% were sensitive. In Thailand, ciprofloxacin resistance among *Campylobacter* species increased from zero before 1991 to 84% in 1995. In addition, azithromycin resistance was found in 7%-15% of *Campylobacter* isolates in 1994 and 1995. Resistance to trimethroprim-sulfamethoxazole has been noted among strains from Egypt.

3.7 *Campylobacter* as cause of travellers' diarrhoea

Campylobacter is an important cause of diarrhoea associated with travel to developing countries. *C. jejuni* is commonly associated with travellers' diarrhoea in expatriates living in Nepal, Thailand and Bangladesh. It has also been the leading cause of travellers' diarrhoea among United States troops in Thailand and Egypt. Furthermore, the organism was the most common bacterial pathogen isolated from Austrian tourists returning from abroad. Ten per cent of diarrhoea in travellers to South Asia is caused by *C. jejuni*. *Campylobacter* were isolated significantly less often in patients who had lived in Thailand for more than 1 year, compared with those who had lived there less than 1 year. In study of Finnish tourists visiting Morocco *Campylobacter enteritis* showed seasonal variation being the leading cause of diarrhoea in winter (28% of cases), but not in autumn (7%). Individuals with travellers' diarrhoea due to *Campylobacter* species tended to have the most severe disease.

3.8 Clinical features

The clinical spectrum of *Campylobacter enteritis* ranges from a watery, non-bloody, noninflammatory diarrhoea to a severe inflammatory diarrhoea with abdominal pain and fever. In Nigerian children, *Campylobacter enteritis* is characterised by history of watery offensive stool lasting under 5 days while acute watery diarrhoea is the major presentation among infants and young children in Bangladesh (Table 2). Watery diarrhoea (97.6% cases) was the most common clinical presentation of patients found to be infected solely by *C. jejuni*/coli in Calcutta, India. In Ethiopia, among children aged 1-5 years, *Campylobacter* was more frequently isolated from those presenting with persistent diarrhoea than from among those with acute illness. Among *Campylobacter*-positive diarrhoeal patients in a survey in Alexandria, Egypt, 69.0% had faecal leukocytes present and 16.3% had bloody stools while dehydration was reported in > 40% of those with *Campylobacter*-associated illness in 8 rural villages, Northeastern Egypt. In South Africa, *Campylobacter enteritis* in children under 2 years was usually mild, without macroscopic blood in the faeces, and prolonged excretion of the organism after acute attacks was not infrequent.

3.9 Guillain-Barré Syndrome

Guillain-Barré Syndrome (GBS) is an acute imflammatory demyelinating polyradiculoneuropathy. *Campylobacter jejuni* infection is most frequently identified with infection preceding GBS. It is reported to occur worldwide. In the developing world sporadic GBS cases associated with *C. jejuni* infection have been reported from China, South Africa and India. Summer epidemics of GBS occur among children and young adults in Northern China and are particularly likely to be associated with *C. jejuni* infection. Lack of reporting of GBS from most developing countries may be due to misdiagnosis especially in polio endemic areas.

Table 1 Isolation rates of Campylobacter from diarrhoea specimens from under-5-year-olds in some developing countries in WHO Regions

WHO Region and Country	Isolation rate (%)
Africa:	
Algeria	17.7
Cameroon	7.7
Central African Republic	10.9
Ethiopia	13.8
Gambia	14.3
Nigeria	16.5
Tanzania	18.0
South Africa	15.4
Americas:	
Chile	13.8
Costa Rica	10.5
Guatemala	12.1
Mexico	12.0
Eastern Mediterranean:	
Egypt	7.7
Pakistan	12.0
Saudi Arabia	4.5
Europe:	
Former Yugoslavia	16.3
South East Asia:	
Bangladesh	17.6
Thailand	13.0
Western Pacific:	
China	10.0
Lao	4.4
Papua New Guinea	12.0

Table 2 Clinical features of patients with diarrhoea associated with C. jejuni in Bangladesh[a] and Nigeria[b]

Symptom	No. of patients with symptom due to C. jejuni in: Bangladesh (n = 164)	Nigeria (n = 15)
Watery diarrhoea	66	15
Abdominal pain	45	0
Bloody stool	17	0
Mucoid stool	61	0
Vomiting	66	2
Fever	50	6
Dehydration	20	3
Malnutrition	ND[c]	4

[a]Adapted from Taylor (1992)
[b]Coker and Dosunmu-Ogunbi (1985)
[c]Not determined

4. Sources of human Campylobacteriosis

The sources of human infection with *Campylobacters* in developing countries include environmental contamination, humans excreting the organisms, or foods.

4.1 Environmental contamination

Wild birds and domestic animals are known as reservoirs for *Campylobacters* and shedding of the bacteria causes contamination of the environment. *C. jejuni* was isolated from 43.6% was from local domestic fowls, followed by goats (33.3%) and sheep (23%) in rural Ghana. Twenty eight per cent of children with *Campylobacter enteritis* in Yaounde were exposed to chickens while 23.8% regularly drank water from streams, which probably were contaminated. In Zimbabwe, *Campylobacter* species were found to be common in chicken faeces collected from the homesteads of the farm workers. *Campylobacter* infections were statistically associated with the presence of live poultry and the lack of piped water in homes in Central African Republics. In Egypt, Campylobacteriosis among infants was positively associated with keeping fowl in the home or having an outdoor source of drinking water. In Chengdu, China, contact with animals a week before being ill and habit of intake of food with hands are risk factors of *C. jejuni*/coli infection of childhood. There is a correlation between strains isolated from human and chickens confirming that chickens are important source of human Campylobacteriosis in developing countries. Biotype and serotypes recovered from animals and birds were also found to be prevalent in strains isolated from clinical sources. Results from a study in Nigeria show that *C. jejuni* biotype I was the prevalent biotype isolated from human and animal sources. Serotypes 2, 4, 29 and 36 were found to be responsible for most *enteritis* in humans, pigs and chickens. These serotypes would be involved in an apparent animal to human transmission routes of *Campylobacter* infection in Nigeria. Pigs and chickens are mostly free-living domesticated animal in Nigeria, thus emphasizing the higher risk to which people are exposed particularly children.

4.2 Humans excreting the organisms

Humans with Campylobacteriosis are potential sources of infection. In developing countries, the mean duration of convalescent-phase excretion of *Campylobacter* organisms after an acute infection is 8 days. In Liberia, heavy environmental contamination with *Campylobacter*, probably of both human and animal faecal origin was observed. Asymptomatic shedding in Egyptian children without diarrhoea was positively associated with a recent diarrhoeal episode. In addition, asymptomatic constant reinfection with new, different serotypes as seen in Mexican patients may be an important source of new infections.

4.3 Foods

Campylobacters present in foods for consumption in developing countries as a result of poor sanitation are an important potential source of infection in humans. In Nairobi, Kenya, *Campylobacters* were isolated from 77% and 40% of retail poultry meat sold in Nairobi, Kenya and Bangkok, Thailand respectively. The serotypes of the organisms isolated in Thailand were similar to those of organisms isolated from humans. In Mexico City a survey of ready-to-consume roasted chicken show that the products are contaminated with *Campylobacters*.

5. Conclusion

The incidence of Campylobacteriosis in developing countries has been estimated from isolation of enteric pathogens in cases of diarrhoea apparently due to lack of surveillance systems. There is thus a need to strengthen the awareness and diagnostic facilities for Campylobacteriosis with a view to setting up national surveillance programs. To this end, national surveys are therefore imperative to determine the epidemiological risk factors, distribution of strains in immunocompromised individuals, seasonal variation, current state of resistance to antimicrobial agents, identification of emerging organisms such as Arcobacter, and the present role of Campylobacteriosis in GBS. There is also an urgent need to strengthen collaboration among researchers in developed and developing countries. These will contribute to understanding the global epidemiology of Campylobacteriosis.

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4. Health burden due to infection with thermophilic *Campylobacter* spp.[1]

1 Extended abstract of article accepted for publication in Epidemiology and Infection

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Infection with thermophilic *Campylobacter* spp. (mainly *C. jejuni*) usually leads to an episode of acute gastro-enteritis. Occasionally, more severe and prolonged diseases may be induced, notably Guillain-Barré syndrome and reactive arthritis. For some patients, the disease may be fatal. We describe the epidemiology of illness associated with thermophilic *Campylobacter* spp. in the Netherlands in the period 1990-1995 and integrate the available information in one public health metric, the Disability Adjusted Life Year (DALY). DALYs are the sum of Years of Life Lost by premature mortality and Years Lived with Disability, weighed with a factor between 0 and 1 for the severity of the illness.

The annual incidence of *Campylobacter* associated enteritis, as measured in a community-based study, is 310,000 cases per year. Approximately 18,000 patients visit their general practitioner (excluding consultations by telephone). A faecal sample is sent to a laboratory and tested positive for *Campylobacter* for 6,800 patients. Only a small fraction of all cases is involved in recognised foodborne outbreaks. The number of fatal cases is highly uncertain, with a most likely value of 30 per year, mainly among the elderly. The incidence of Guillain-Barré syndrome in the Netherlands is approximately 180 cases per year, of which 60 are induced by infection with *C. jejuni*. Of these, 50 are severely affected (i.e. not able to walk independently). Mortality is low (1 case per year) but there is considerable residual disability; as much as 30% of the severely affected patients did not fully recover but continue to suffer from functional limitations. The incidence of *C. jejuni* related reactive arthritis in the Netherlands is also highly uncertain, with a most likely value of 6500 cases per year.

Severity weights for acute enteritis and the different stages of Guillain-Barré syndrome were obtained by panel elicitation, using protocols developed for the Dutch Public Health Status and Forecast Study (PHSF). Duration of disease and life expectancy of fatal cases were obtained form different epidemiological studies.

Combining this information, the health burden of illness associated with thermophilic *Campylobacter* spp. in the Dutch population can be estimated. Point estimates are shown in Table 1. The mean health burden is estimated as approximately 1400 DALY per year.

Uncertainty and variability in the epidemiological information are explicitly taken into account in the analysis by Monte Carlo simulation, and by sensitivity analysis. The Monte Carlo simulation results in a 90% confidence interval between 900-2000 DALY per year (see Figure 1).

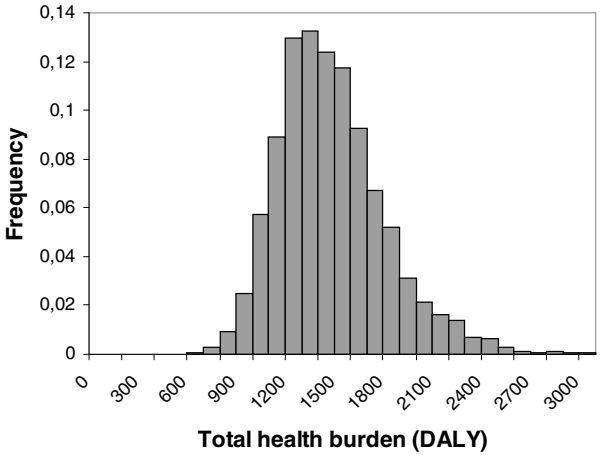

Fig. 1. Distribution of estimated health burden by infection with thermophilic Campylobacter spp. in the Netherlands

Table 1. Health burden due to infection with thermophilic Campylobacter spp. in the Netherlands[1]

a. Morbidity

	Number of cases	Duration (years)	Severity weight	YLD
Gastro-enteritis				
General population	311,000	0.014	0.067	291
General practitioner	17,500	0.023	0.393	159
Guillain-Barré syndrome				
Clinical phase	58.3	1	0.281	16
Residual symptoms	57.0	37.1	0.158	334
Reactive arthritis	6570	0.115	0.210	159
Total				959

b. Mortality

Population	Number of cases	Life expectancy (years)	Severity weight	YLL
Gastro-enteritis	31.7	13.2	1.00	419
Guillain-Barré syndrome	1.3	18.7	1.00	25
Total				444

c. Health burden

Population	YLD	YLL	DALY
Gastro-enteritis			
General population	291	419	710
General practitioner	159		159
Guillain-Barré syndrome			
Clinical phase	16	25	41
Residual symptoms	334		334
Reactive arthritis	159		159
Total	959	444	1403

[1] Based on mean values of the estimated annual incidence, the severity weight and the duration

Sensitivity analysis evaluated the effects of alternative model assumptions on the health burden estimates, see Tables 2 and 3, and Figure 2.

The main determinants of health burden are acute gastro-enteritis in the general population (290 DALY), gastroenteritis-related mortality (420 DALY) and residual symptoms of Guillain-Barré syndrome (330 DALY). The health burden associated with gastro-intestinal pathogens may be underestimated if only diarrhoeal illness is accounted for. The most important causes of health burden affect patients that are not usually seen in clinical settings. Most detailed data are available from clinical studies, but these relate to diseases or disease stages that only have a small contribution to the overall health burden. Thus, active surveillance for gastro-intestinal pathogens, based on population studies is preferred above passive surveillance based on clinical reports. Comparison with results from a national study shows that the health burden of *Campylobacter* infection is similar to diseases such as meningitis, sepsis, upper respiratory infections, stomach and duodenal ulcers, Down syndrome, violence and accidental drowning.

Table 2. Alternative assumptions used in sensitivity analysis

Scenario #	Description
1	Alternative case-definition for gastroenteritis in population study
2	Higher severity weight for gastro-enteritis
3	No correction for non-response in GP surveillance
4	High incidence estimate for Guillain-Barré syndrome
5	Probability of Guillain-Barré syndrome according to US results
6	No correction for sensitivity serology in antecedent infections for Guillain-Barré syndrome
7	Severe cases of reactive arthritis only
8	Severity weights from regression model
9	Variability in case-fatality ratio of gastroenteritis
10	Fatal cases of gastroenteritis have lower life expectancy or reduced quality of life

Table 3. Effect of alternative assumptions on DALY estimates for individual disease endpoints and total over all endpoints

Endpoint	DALY in scenario (% change)								
	Base-line	1	2	3	4	5	6	7	8
GE population	710	562 (-21%)	810 (+14%)						1071 (+51%)
GE general practitioner	159			94 (-41%)					162 (+2%)
GBS clinical	41				128 (+208%)	73 (+75%)	31 (-26%)		38 (-8%)
GBS residual	334				1031 (+208%)	586 (+75%)	247 (-26%)		224 (-33%)
Reactive arthritis	159	128 (-20%)						9 (-94%)	
Total	1403	1224 (-13%)	1503 (+7%)	1354 (-3%)	2186 (+56%)	1686 (+20%)	1305 (-7%)	1253 (-11%)	1653 (+18%)

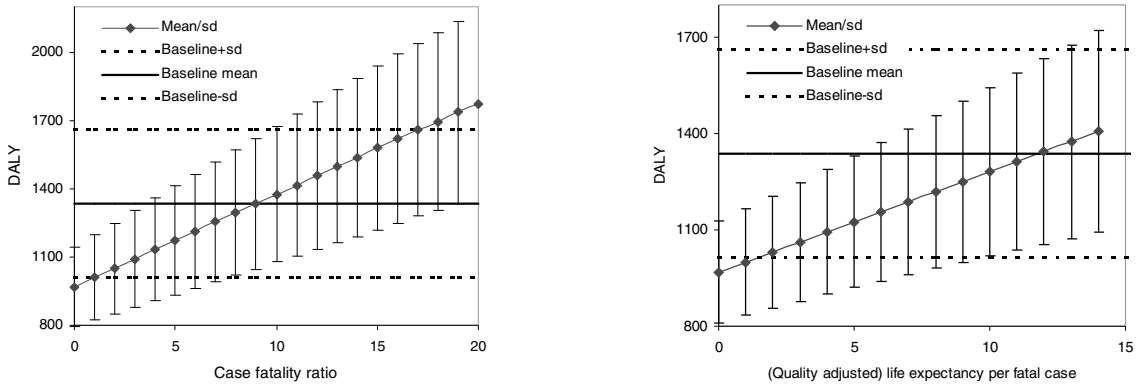

Fig 2. Effect of the case-fatality ratio (left) or the life expectancy or quality of life of fatal cases (right) of gastro-enteritis on the total health burden and the associated uncertainty

5. What have we learned about *Campylobacter coli/ jejuni* from bacteriologival typing studies?

Stephen L. W. On

Introduction

The species *Campylobacter jejuni* and *C. Coli* are widely regarded as the most frequent bacterial causes of diarrhoea in humans worldwide (1,2). They are widely distributed and may be found in production, domestic, and exotic animals, and in the environment (1,2). It is partly due to their ubiquitous distribution that there remain myriad questions concerning their epidemiology. It is with these questions in mind that a variety of phenotypic and genotypic methods for epidemiological typing have been developed over the past two decades. The purpose of this minireview is to summarise briefly what we have learned about *Campylobacter* epidemiology from the results of typing studies and to use these data to make informed presumptions about likely sources and modes of transmission.

1. Typing methods: a brief note

The efficacy of available typing methods will be discussed elsewhere in the meeting. However, it is necessary to briefly discuss them and distinguish them into two broad groups for the purpose of perspective. Phenotypic typing methods include biotyping, serotyping, phage typing, cellular fatty acid profiling, and protein electrophoretyping. Results are based upon the expression of genetic information, usually reflect only a small percentage of the genetic content and may be affected by environmental conditions (3). Consequently, phenotypic typing systems are less discriminatory than those based on direct examination of DNA, may not accurately reflect close strain relationships and may be unstable. Nonetheless, since the criteria are generally determinative, the methods can be used to determine specific, definitive "types".

Genotypic methods involve direct examination of the genetic content of living organisms. They examine for changes in the DNA sequence of specific single (eg. flagellin) - or multiple (i.e. MLST) gene loci, or over the whole genome (eg. PFGE, RAPD, AFLP) to determine differences or similarities between strains. These differences can often be quantified but results may not necessarily reflect the geneaology of the strains correctly, where differences are detected. However, by direct examination for genetic polymorphisms, environmental factors are less influential and the results obtained are generally regarded as being more stable and discriminatory than phenotyping methods (4). Thus, where strains match in a given sequence or genetic banding pattern, there is increased security in making the assumption that they are related. Nonetheless various genetic phenomena can and does affect the genetic content of bacteria, sometimes resulting in mis-interpretation of data (5-7). The efficacy of both phenotypic and genotypic typing methods, and phenomena affecting the results of DNA-based techniques are authoritatively covered elsewhere in several recent reviews (3, 4, 8).

1. Campylobacter and outbreaks

By use of various typing methods, it is well established that *Campylobacters* can be disseminated from a common source and may cause outbreaks of gastrointestinal disease in humans. Outbreaks are normally identified as a sudden increase in incidence of disease during a short time period in a limited geographic area. They are usually caused by point contamination of food or water and a single strain is normally found responsible for the ensuing disease. The limited chronologic and geographic characteristics of an outbreak make their confirmation by typing methods relatively straightforward. Almost any typing method can be used to identify outbreak-related strains, although if isolates belong to a

relatively common type in the region of interest, accurate identification of the point of origin, and assessment of the magnitude of the outbreak, can be problematic.

The application of typing methods to outbreaks have confirmed several vehicles as sources of infection. In this way, the consumption of contaminated (raw or underpasteurised) cows or goats milk (9-12), water (surface and ground) (12-15), chicken (16, 17); and close contact with cattle (18), dogs (19), and coyotes (20), can be considered as documented sources of outbreak infection. Interestingly, few cases of foodborne outbreaks reported in the literature appear to have the presumed source of infection conclusively linked to the outbreak by use of strain typing. This is likely to be a result of the suspected source being unavailable for examination, having been either consumed, or unsuitable for *Campylobacter* culture due to extended cold storage, than a lack of importance as a vehicle for outbreak infection.

Closed animal communities can be viewed as models of outbreak infection. Some studies in poultry have demonstrated the same *C. jejuni* strain in each of the outgoing and incoming flocks, suggesting that some strains may persist in the environment between flock rotations and cause subsequent contamination (21), or that transmission between neighbouring flocks occurs (22). In the latter case, beetles are a possible vector (23). Type homogeneity in flocks has also been used to argue for vertical transmission (24) but this remains hypothetical. The trade of infected live animals can contaminate birds free from *Campylobacters*, as documented in an outbreak of *Campylobacter*-associated hepatitis in ostriches (25). A contaminated water supply was strongly implicated as the source of infected poultry in one study (26) and wildliving animals (eg. deer, hedgehogs) may also represent a reservoir of infection (27). In any case, the introduction of *Campylobacter* to poultry often results in its rapid spread throughout the flock (21). In pigs, maternal transmission has been suggested by typing studies (28), although the results ultimately can be viewed as equivocal since wholly unrelated strains also shared the same genetic type. Contaminated lake water was proposed as the source of *C. jejuni* in grazing cattle in one investigation (29).

2. *Campylobacters* and sporadic infection: A brief introduction

Most reported cases of *Campylobacter* infection are sporadic and no single point of infection is identified (1, 2). This does not mean that no focal origin of infection exists but simply that it is not detected. For example, a single batch of contaminated food from one warehouse could be disseminated to a supermarket chain and be sold to consumers across the country without ever being identified as the source of infections reported locally. At present we lack the necessary infrastructure and indeed methods to rapidly and effectively identify such outbreaks. As a consequence, using typing data to investigate the sources and routes of transmission for sporadic Campylobacteriosis is entirely speculative.

Serotyping and, to a lesser extent, phage typing, has for almost two decades been used as a tool to infer relationships between sporadic human isolates and those isolated from production animals, pets and foodstuffs (2, 3, 30). Each of the latter sources have been implicated as vehicles for human *Campylobacter* infection, but the limited discriminatory power of serotyping, as well as the relative predominance of some serotypes, makes an accurate assessment of the relative contribution (if any) of the aforementioned sources to human disease impossible. Later studies have used molecular typing methods to investigate the same issue, ultimately with the same equivocal result. Some molecular methods offer rather limited discriminatory potential (eg. ribotyping, flagellin gene polymorphisms) (eg. 31, 32), whilst even those examining whole-genome polymorphisms (eg. PFGE) often differ with other data from additional typing methods (31, 32). The additional problems associated with DNA-based profiles changing as a result of genetic instability (8) prevent firm conclusions about the relationship of any two strains from apparently unrelated sources from being made under these circumstances.

Campylobacters and sporadic infection: realistic presumptions from multifactoral typing analyses

Making reasonable presumptions about the possible relatedness or significance of two epidemiologically unrelated strains is dependent upon the confidence with which one can say "they are the same". Because of the problems mentioned above, a strict polyphasic definition of strain or clonal identity is required to provide that confidence. Therefore, presumptions about the clonal/epidemiological origin of any two strains must be based on multifactoral typing data. This is a rational application of the laws of probability and not dissimilar to a classical definition of a bacterial clone given in 1983 (33). In short, the more characteristics shared by two strains, the more probable it is that they share a common (i.e. clonal) origin. The characteristics should be independent of one another and ideally include both phenotypic and genotypic data. However, reasonable confidence of a clonal relationship between two

strains can be obtained where whole-genome polymorphisms are determined with at least two restriction site loci. The latter approach has been used to identify clones of *C. jejuni* in humans and chicken samples in Finland (34). A combination of ribotyping with two different enzymes and PFGE-DNA profiling of two common serotypes in the United Kingdom identified common *C. jejuni* strains in humans, poultry, cattle, sheep and a dog (35). Similarly, combined serotyping, ribotyping, PFGE-DNA typing and PCR-*fla* gene typing identified the same *C. Coli* strain in humans and sheep in England (36). Serotyping combined with PFGE-DNA profiling with each of four restriction enzymes demonstrated common types of *C. jejuni* in humans, poultry, cattle and swine in Denmark (37). These findings concur with results of another Danish study using six different phenotypic and genotypic methods to examine 100 strains of *C. jejuni*, where strains from humans, cattle and poultry proved indistinguishable with all methods (38).

We can reasonably conclude from these studies that poultry, cattle, pigs, sheep, dogs and/or their products, may account for at least some of the sporadic human infections reported in these countries. The data are insufficient to prove that any or all of the aforementioned production animals are sources of human infection: without epidemiological information, it is impossible to identify the direction of infection pressure (i.e. animals to human, or human to animal). Nonetheless when case-control studies are taken into account (38, 39), the presumption that poultry, cattle, sheep, pigs and dogs are sources of sporadic human *Campylobacter* infection is wholly credible.

By contrast, *C. jejuni* strains associated with the neurological disorder Guillain-Barré syndrome (GBS) and the closely related Miller-Fisher syndrome (MIS) belong to a range of serotypes and are also genotypically diverse (40). The determining factor governing development of the disease following infection appears to be expression of certain surface lipopolysaccarides of *C. jejuni* that mimic human gangliosides, thus initiating an autoimmune response in the host (41). Although certain serotypes (notably 0:19) have historically been associated more frequently with the onset of GBS (41), more recent data demonstrate a less marked serotype-specific association, with neither serotyping or genotyping suggesting the existence of GBS/MIS-specific strains (40).

Evidence for internationally disseminated clones

Several multifactoral typing studies have identified strains isolated from different countries at different times, and sometimes from different hosts, that nonetheless share several phenotypic and genotypic features. In some cases the time difference between a pair of strains spans two decades. The conservation of phenotypic and genotypic characters in strains of species that are known to exhibit considerable phenotypic and genotypic heterogeneity, and with established precedents for spontaneous genetic change, is strong evidence for the existence of genetically stable clones. Such strains have been documented by polyphasic typing criteria in the USA and Canada (42), the UK, Canada and New Zealand (43), and England and South Africa (35)for *C. jejuni*; and in the USA and Canada (42) and England and Portugal (36) for *C. Coli*. The importance and significance of these strains is impossible to determine in the absence of further studies of their virulence and/or transmissibility characteristics.

Travel is often cited as a significant risk factor to humans for acquiring *Campylobacter* infection (2). The aforementioned data may infer the cross-border transfer of some infections acquired abroad, although there are insufficient results from strains isolated in popular holiday destinations (from a European perspective, much of southern Europe) to properly evaluate this hypothesis. Caution is especially pertinent given that there is some evidence to suggest that *Campylobacter* types (44) and even species distribution (45) in developing countries may be significantly different from those in developed countries, from which the above genetically stable clones have been documented.

Summary

Typing studies can authoritatively document that contaminated water, milk, and chicken, and close contact with cattle, dogs, and coyotes, are sources of *Campylobacter* outbreaks.

Typing studies can document with a reasonable degree of security that poultry, cattle, pigs, sheep, dogs and/or their products are sources of sporadic *Campylobacter* infection in humans, when other results (e.g. case-control studies) are considered.

Typing studies demonstrate that some genetically stable strains that are chronologically, ecologically and geographically distinct are extant. These data may suggest the importance of travel as a means of widely disseminating different strains.

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6. What have we learned about *Campylobacter coli/ jejuni* from analytical epidemiological studies?

Kåre Mølbak

See working paper No. 11

7. International Risk Assessment of Microbiological Hazards in Foods

Dr Allan Hogue

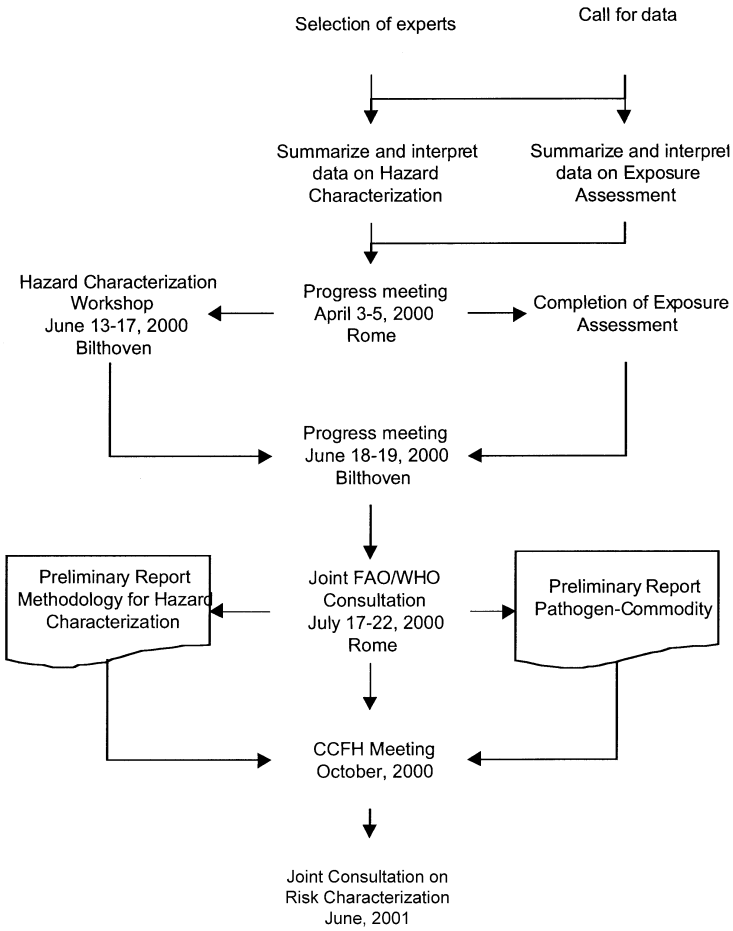
The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) of the United Nations have jointly developed a process for international risk assessment of microbiological hazards in foods. This process incorporates the essential principles of transparency, excellence, and independence of the opinions delivered. These principles are assured through peer review, stakeholder input, and documentation.

The process for international risk assessment of microbiological hazards was developed to provide the scientific recommendations requested by the Codex Alimentarius Commission. At the 32nd meeting of Codex Committee on Food Hygiene in 1999 the Committee developed a list of pathogen-commodity combinations that require expert risk assessment advice. WHO and FAO planned a two year program of work to provide the Committee with the advice needed for two priority pathogen commodity combinations: *Listeria monocytogenes* in ready to eat foods and *Salmonella* spp. in broilers and eggs.

WHO and FAO developed a transparent process for the selection of experts. We sent a call for specialists in microbiology, epidemiology, mathematical modelling, public health, food technology, human medicine, veterinary medicine, and risk assessment to all member countries. A panel of four reviewers (one from WHO, one from FAO, and two independent) reviewed the qualifications of the applicants and produced a roster of about 150 qualified applicants to participate in activities. These activities include: drafting risk assessment reports, reviewing the work of the drafting groups, and participating in expert consultations. FAO and WHO want to ensure that the pool of experts includes a diversity of viewpoints and includes representatives from all geographic regions of the world including both developing and developed countries.

We organized drafting teams composed of ten internationally recognized experts in risk assessment. These teams drafted reports on hazard identification, exposure assessment, and hazard characterization for three commodity-pathogen groups: *Salmonella* spp.

Process of Operation for International Risk Assessment of Microbiological Hazards in Foods



in poultry and eggs and *Listeria monocytogenes* in ready-to-eat foods. These reports were presented to the Joint FAO/WHO Expert meeting on Microbiological Risk Assessment (JEMRA) which met in Rome in July 2000. This consultation reviewed the work and provided expert review and comment on the reports. Preliminary reports on *Salmonella* spp. in poultry and eggs and *Listeria monocytogenes* in ready-to-eat foods were delivered to the CCFH at their meeting in October. In 2001 drafting groups will complete the risk characterization phase. Comments on the report will be solicited from stakeholders and the general public through the internet. The report will be also be peer reviewed at an expert consultation in 2001. The final reports will be delivered to the CCFH in October 2001.

WHO and FAO will initiate new work next year on *Campylobacter jejuni* in broilers and *Vibrio parahaemolyticus* in shellfish using the same process. In November 2000 we will issue a call for experts in the pathogen commodity combinations and organize drafting groups. The risk assessments will be completed in October 2002.

8. Epidemiology of other *Campylobacter* species

Albert J. Lastovica
Mark E. Engel

1. Introduction

Traditionally, more than 99% of *Campylobacter* strains isolated and identified in cases of human disease have been *C. jejuni* subsp. *jejuni* or *C. Coli*. The prevalence and disease potential of other, non-*jejuni/coli Campylobacter* species is beginning to be appreciated, especially in areas where they are commonly isolated. A recent review article (1) details the sources, prevalence, and disease associations of these other *Campylobacter* species.

Isolation procedures originally developed for *C. jejuni/coli* and currently used in many diagnostic laboratories may not support the growth of other, potentially pathogenic non-*jejuni/coli Campylobacter* species. These organisms may be fastidious, requiring special atmospheric and temperature conditions, or be unable to tolerate the antibiotics commonly included in selective media plates, or the incubation period is not sufficiently long enough. An efficient procedure, the "Cape Town Protocol"(2), for the isolation of *Campylobacters* from stool specimens without the use of selective media has been developed and successfully used at the Red Cross Children's hospital, Cape Town, for the last decade. This method involves filtration of stools through a membrane filter onto antibiotic free blood agar plates, and subsequent incubation in an H_2-enhanced microaerobic atmosphere. With a minor modification of this protocol, eleven species of *Campylobacter* or *Helicobacter* have been isolated from the blood cultures of paediatric patients.

To illustrate the difficulties of isolating, characterising and determining the pathogenic potential of non-*jejuni/coli Campylobacter* species, we will draw on our experiences at the Red Cross Children's hospital. For the period Dec 1977 to Sept 1990, Skirrow's and other selective media were used in the diagnostic microbiology laboratory at the Red Cross Children's hospital for the isolation of *Campylobacter* from stools. *Campylobacter* species/subsp. that were isolated were *C. jejuni* subsp. *jejuni*, Skirrow biotypes 1 and 2, *C. jejuni* subsp. *doylei*, *C. Coli* and *C. fetus* subsp. *fetus*. With the introduction of "the Cape Town Protocol", in Oct. 1990, the numbers of species/subsp/biotypes isolated rose to 17 (Table 1). Concurrently, the numbers of stool cultures positive for *Campylobacter* and related organisms rose to 21.8% from 7.1% previously obtained with selective media.

2. Methods

Isolation of strains was accomplished by the "Cape Town Protocol". Phenotypic testing was done by standard procedures. If infection by more than one species was suspected, considerable care was taken to separate the domed colonies of *Campylobacter* from the spreading, non-colonial growth of *Helicobacter fennelliae* or *Helicobacter cinaedi* on the primary plate. Data from culture-confirmed patients admitted to the Red Cross Children's hospital was transferred from laboratory bench books to an epi-info database. Microbiological and other features of infection with the various species isolated were examined in diarrheal and bacteremic paediatric patients in an attempt to further define the characteristics of disease.

3. Results

3.1 General

From October 1990 to August 2000, a total of 4, 260 strains were isolated from the diarrhoeic stools of paediatric patients attending the Red Cross Children's hospital (Table 1). The gender distribution was M:F=1.24:1, a finding consistent with studies conducted elsewhere. The ages of the patients ranged from 1 day to 13 years of age, with

a median of 18.9 months. Clinical characteristics as regards the severity of diarrhoeal symptoms and consistency of the stool were similar for all species isolated including *C. jejuni* subsp. *jejuni*, a universally acknowledged pathogen. *C. jejuni* subsp. *jejuni* with its prevalence of 31%, was the most prominent species, followed by *C. concisus* (24%), *C. upsaliensis* (23%), *C. jejuni* subsp. *doylei* (9%), *H. fennelliae* (6%), and *C. Coli* (3%). Prevalence of the additional species isolated is indicated in Table 1. Based on differences in colony morphology on primary isolation and subsequent biochemical and serological confirmation, 16.2% of the stools of South African paediatric gastro-enteritis patients had multiple isolates of 2 to 5 species. *C. upsaliensis* was frequently co-isolated with other *Campylobacters* such as *C. jejuni* subsp. *jejuni*, *C. jejuni* subsp. *doylei* and particularly with *H. fennelliae*.

The acknowledged pathogens *C. jejuni* subsp. *jejuni* and *C. Coli* only formed about 1/3 of the total range of *Campylobacter* species isolated over a decade in this paediatric patient population (Table 1). This is in distinct contrast to recently published reports where *Campylobacter jejuni* has been the primary, or only *Campylobacter* species isolated from the diarrhoetic stools of paediatric patients (3,4).

Table 1. Distribution of Campylobacter and related species isolated from 20, 458 diarrhetic stools of pediatric patients at the Red Cross Children's Hospital, Cape Town, South Africa, from Oct. 1, 1990 to August 31, 2000.

Species/subspecies	No	%
C. jejuni subsp. jejuni *	1 320	30.99
C. concisus	1 013	23.78
C. upsaliensis	986	23.15
C. jejuni subsp. doylei	388	9.11
H. fennelliae	266	6.24
C. coli	121	2.84
C. hyointestinalis	53	1.24
H. cinaedi	42	0.99
†CLO/HLO	35	0.82
Arcobacter butzleri	16	0.38
C. fetus subsp. fetus	8	0.19
C. sputorum biovar sputorum/C. lari	4	0.09
C. curvus/C. rectus	4	0.09
"H. rappini"	4	0.09
Total	**4,260**	**100.00**

*Biotypes 1 and 2 of Skirrow and Benjamin
†CLO/HLO = *Campylobacter* or Helicobacter-like organisms

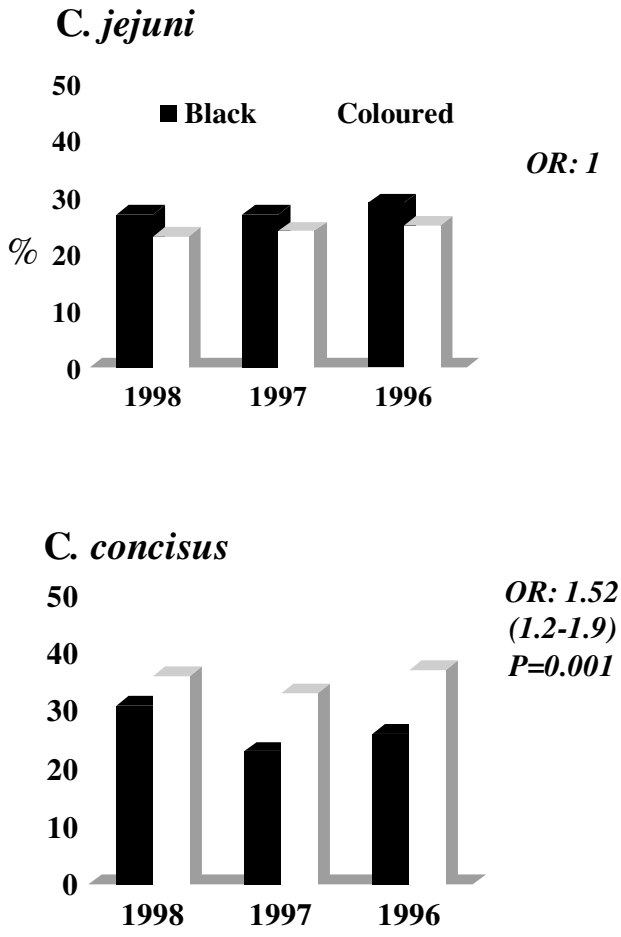


Fig 1. Distribution of C. jejuni subsp. jejuni *and* C. concisus *isolates among hospitalised, coloured and black South African children with gastro-enteritis*

3.2 *C. concisus*

Campylobacter concisus was the second most prevalent *Campylobacter* species isolated. On examination of clinical and other features of infection in an attempt to further define the characteristics of disease associated with *C. concisus* as compared to the recognised pathogen, *C. jejuni* subsp. *jejuni*, it was found that *C. jejuni* subsp. *jejuni* predominated in children under 12 months of age, while *C. concisus* was more prevalent in children 1 year or older. Seventy-five % of the stools of *C. concisus* patients were loose, and 21% were watery, while 4% were formed. Diarrhoea was present in 66% of the patients, while fever or vomiting was present in 5%. Two % of the patients were HIV+ or immunocompromised.

In a subset of 2, 170 *Campylobacter* strains isolated from the period January 1994 to December 1998, the number of isolates of both *C. concisus* and *C. jejuni* during the 3-month period towards the end of South African summer (Feb to April) was notably higher than during the corresponding

3-month winter period (May to July) (45% vs. 14%, p< 0.01). Clinical characteristics such as the severity of diarrheal symptoms and the consistency of the stool (watery vs. loose) were similar for both species examined. *C. concisus* was more likely to be associated with coloured rather than black children (Fig 1), (OR=1.69, p=0.001). For *C. jejuni* this association was (OR=1, p=0.4) and thus the association cannot be readily explained by age or gender differences. Factors such as genetic susceptibility, dietary habits or geographical location may be involved and further research is required to clarify these observations and to provide additional data for the elucidation of the pathogenic properties of *C. concisus*. While over a thousand stool isolates of *C. concisus* were recorded (Table 1), only one blood culture strain from an 18 day old infant was isolated (Fig 2), strongly suggestive that *C. concisus* is not invasive from the gut.

3.3 *C. upsaliensis*

C. upsaliensis was the third most prevalent *Campylobacter* species isolated from the diarrhoeic stools of paediatric patients (Table 1). Nine hundred and eighty-six strains of *C. upsaliensis* formed 23% of the 4, 260 *Campylobacters* isolated during a 10 year period. Clinical conditions of the patients included diarrhoea 62%, vomiting or fever 3%, loose stools 72% and 18% with watery stools. Twelve % of the patients were HIV+, or had immunodeficiency or nutritional problems.

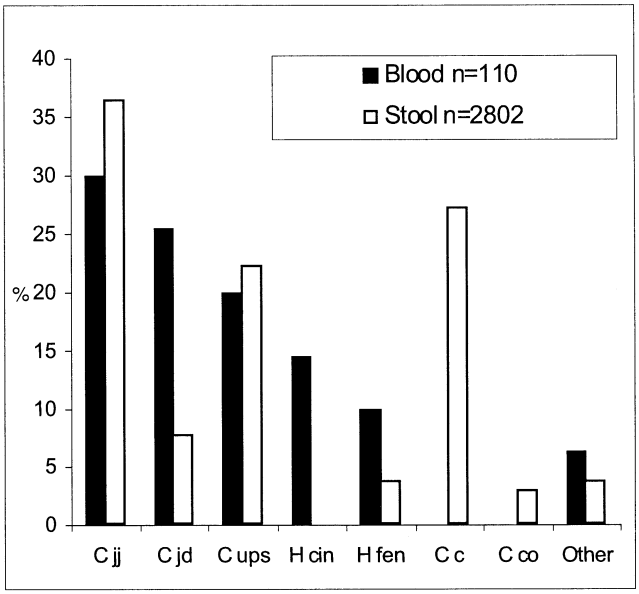


Fig 2. Campylobacter species isolated from pediatric blood & stool cultures in Cape Town, South Africa (1990-2000)[1].

C jj: C. jejuni subsp jejuni; C jd: C. jejuni subsp doylei; C ups: C. upsaliensis; H cin: H. cinaedi; H fen: H. fennelliae; C c: C. concisus; C co: C. coli

[1] Sole pathogen detected.

C. jejuni predominated in children less than 12 months of age while *C. upsaliensis* was more common in older children.

Fig. 2 indicates that *C. upsaliensis* is almost equally found in blood (20%) as stool cultures (23%). Most patients with *C. upsaliensis* bacteremia have other, serious underlying medical conditions, and bacteremia may have been secondary to intestinal infections.

3.4 *Campylobacter* subsp. *doylei*

Three hundred and eighty-eight strains of *C. jejuni* subsp. *doylei* comprised 9.11% of a total of 4, 260 *Campylobacter* and related species (Table 1).

Seventy-two percent of the diarrhoeic children had loose and 25% had watery stools. Sixty-four % of the patients displayed diarrhoea, and 5% were vomiting or feverish. Ten % of the children had nutrition-related problems and 2% had anaemia. Of 110 *Campylobacter* blood culture isolates, 28 (25%) were *C. jejuni* subsp. *doylei* (Fig 2) Fourteen of these 28 children had diarrhoea, often chronic, suggesting that intestinal infection preceded systemic infection. Twenty of these 28 patients were suffering from severe protein deficiency diseases such as marasmus and kwashiorkor. *C. jejuni* subsp. *doylei* comprised less than 10% of the *Campylobacters* found in stool (Table 1), but formed 25% of the *Campylobacter* blood cultures seen at the same paediatric hospital (Fig 2). This observation suggests a pathogenic, possibly invasive, role for *C. jejuni* subsp. *doylei*.

3.5 *Helicobacter fennelliae* and *Helicobacter cinaedi*

Forty two *H. cinaedi* and 266 *H. fennelliae* strains were isolated from gastro-enteritis patients over a decade (Table 1). *H. fennelliae* and *H. cinaedi* were frequently co-isolated with *C. jejuni* subsp. *jejuni* or *C. jejuni* subsp *doylei.* The median age of the infected patients was 19 months for *H. cinaedi* and 23 months for *H. fennelliae* (range 2 weeks-11 years). Sixty-four percent of these patients displayed diarrhoea, 1% had vomiting and 2% fever. Ten % of the children had pre-existing conditions such a positive HIV status, or nutritional complaints. *H. fennelliae* and *H. cinaedi* are much more likely to be associated with blood rather than stool infection, and this may imply an invasive role for these organisms (Fig. 2).

3.6 *Campylobacter hyointestinalis*

Fifty-three strains of *C. hyointestinalis* were isolated from diarrhoeic stools (Table 1). The median age of the *C. hyointestinalis* patients was 29 months (range: 1 month to 7 years). 71% of the patients displayed diarrhoea, none displayed

vomiting or fever. One blood culture isolate of *C. hyointestinalis* was obtained from a 22-year-old male after a bone marrow transplantation operation.

4. Conclusion

The results presented here represent findings from a single hospital over a 10 year period. The prevalence, and hence, the disease potential of non-*jejuni/coli Campylobacter* species elsewhere is largely unknown. Our study indicates putative pathogens such as *C. upsaliensis*, *C. concisus*, and *C. jejuni* subsp. *doylei,* when compared to recognised pathogens such as *C. jejuni* subsp. *jejuni* or *C. Coli,* display the same clinical presentations in South African paediatric gastro-enteritis patients.

C. upsaliensis is as prominent among blood culture as stool isolates. *H. cinaedi*, *H. fennelliae*, and in particular, *C. jejuni* subsp. *doylei* are much more prevalent as blood culture than stool pathogens. This is suggestive of an invasive capability for these species.

New species of the genus *Campylobacter* and related genera are being identified on a regular basis. Many of these "other" *Campylobacters* may play a greater role in causing human and animal disease than previously recognised. As methods originally formulated for the isolation of *C. jejuni* will often fail to support the growth of non-*jejuni/coli Campylobacter* species, these fastidious organisms are most likely under-detected in clinical specimens. Appreciation and application of the correct protocol is essential for the isolation of non-*jejuni/coli Campylobacte*r species for prevalence, epidemiological, surveillance, and other studies.

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9. Major risk factors for human Campylobacteriosis - An overview

Robert V. Tauxe

Extended abstract of presentation

Defining the risk factors for infection is critical to developing appropriate targeted prevention strategies. These may include risk factors associated with the human host, with the pathogens itself, and with specific environmental exposures, including food, water and animals.

Host factors play a role in defining who is at risk for *Campylobacter* infection. There is evidence of acquired immunity, for example among persons who routinely consume raw milk. Persons with immunocompromise appear to be at higher risk for infection. The complication of Guillain Barre Syndrome (GBS) may be more likely among the elderly, though specific host factors remained to be defined for this complication.

Pathogen specific risk factors are also poorly defined. No major differences in virulence among *C. jejuni* strains are apparent epidemiologically. A strong association between infection with serotype O:19 and the subsequent development of GBS reported from Asia has been difficult to substantiate with data from other continents.

Environmental risk factors and exposures have been identified in case-control studies of sporadic cases and in outbreak investigations, and appear to be somewhat different for sporadic cases than for outbreaks. The vast majority of *Campylobacter* infections occur as sporadic individual infections. For non-travel-associated infections, the most consistent risk factor in a number of case-controls studies in the United States New Zealand and Europe has been consumption of, cross-contamination from or contact with raw or undercooked poultry, accounting for a range of approximately 10% to 50% of cases. In early U. S. studies, chicken prepared in the home, and direct contact with raw chicken during the preparation process were important risks. In Great Britain, New Zealand, and in the most recent U.S. case-control study, the risky poultry was specifically that eaten outside of the home, i.e. chicken prepared in restaurants. In New Zealand, the consumption of chicken livers and other organ meats was also specifically associated with illness. Other foods have been identified as sources of infections, including consumption of sausages at a barbecue in Norway, and consumption of barbecued red meat in Denmark.

Drinking untreated water is also a specific risk factor in the majority of studies, including water from streams or ponds, shallow private wells, and rainwater collected from roof gutters. Raw milk has been identified as a risk factor in the largest studies, and may represent an appreciable fraction of the total in populations that frequently drink raw milk. Contact with animals, particularly pet dogs and cats, has been repeatedly identified as a risk factor for sporadic infections.

Direct information about the source of *Campylobacter* infections in the developing world has been more difficult to obtain. In a trial of household water chlorination in Bolivia in 1996, we noted that *Campylobacter* infections were eliminated from the intervention group, while they were the most common diagnosed cause of diarrhea in the control group; this occurred in a population drinking water from shallow wells in yards where chickens were ubiquitous.

In a recent case-control study completed at FoodNet sites in the United States, analysis of cases of fluoroquinolone resistant infection and matched healthy controls showed that these infections were strongly associated with two independent exposures: international travel, and consumption of chicken and turkey prepared in commercial food establishments. International travel was reported by 42% of persons with FQ-resistant infections. Veterinary use of fluoroquinolones in poultry in the United States may explain the recent rise in domestically acquired ciprofloxacin-resistant

Campylobacter infections in humans associated with poultry consumption.

Common source outbreaks account for a very small fraction of reported cases. Outbreaks due to raw milk and untreated drinking water have been the most common sources of outbreaks in the past, but are decreasing in the last decade in the United States; this may be because of increased disinfection of ground and surface water before distribution and public health warnings about the hazards of raw milk consumption, particularly on farm visits by school children. A variety of other foods have been associated with outbreaks of *Campylobacter* infection, that probably represent cross contamination in the kitchen from raw poultry or other sources. An ill food preparer has been identified as the source in rare instances. Food vehicles can be unusual; for example, it was recently shown that *Campylobacter* can survive for long time periods after inoculation into butter.

It is noteworthy that despite the low inoculum demonstrated in voluntary challenge trials, there is little evidence that *Campylobacter* is frequently spread directly from human feces. Unlike *Shigella*, *Campylobacter* does not often produce secondary infections in households, and has not emerged as a common cause of prolonged outbreaks in child care centers or residences for mentally retarded persons. *Campylobacter* infection rates in men who have sex with men may be no higher than in heterosexual men of the same age. This relative lack of human-to-human transmission is unusual for a low inoculum infection, and suggests that *Campylobacter* may be less infectious after passage through a human host than it is after passage through a broiler chicken or a contaminated water source.

It is also noteworthy that unlike *Salmonella*, persons who for other reasons take antimicrobials that might affect the gut flora have not been shown to be at higher risk for Campylobacteriosis. This suggests that the indigenous flora may have little impact on immunity to infection.

Prevention of *Campylobacter* infections will require efforts to reduce exposure to several different sources of infection, including contaminated poultry, untreated drinking water and unpasteurized milk. This implies a multi-pronged approach to prevention is needed, that may require different emphases in different locations, depending on which sources predominate.

10. Emergence of antimicrobial resistance in *Campylobacter*: The consequences for incidence, clinical course, epidemiology and control

Heriberto Fernández

Since the first isolations from diarrheal cases at the beginning of the 70's, the understanding of the role of *Campylobacter* jejuni and *C. Coli*, as well as of other *Campylobacter* species, as enteropathogenic agents for human beings, has greatly increased. They are considered among the most frequent organisms causing diarrheal illness in Europe, the United States and other industrialized countries. In many underdeveloped countries, *C. jejuni* and *C. Coli* seems to be hyperendemic but, in others, they are commonly considered as the second or the third cause of diarrhea, especially among infants.

However, despite the high global frequency of *Campylobacter* isoltions observed in developed and developing countries, there are several differences that could be observed among both types of countries with regard to the epidemiological and clincial aspects of these bacteria, as well as to the accurate perception of the sanitary autorithies of the real magnitude of the complex public health problem they represent.

I would like to remark some of these differences observed in South American countries but first, I consider necessary to point out that a vast and valuable information on bacteriological, pathogenical, clinical and epidemiologial aspects of Campylobacteriosis has been produced in this region which is available in the biomedical bibliografic databases MEDLINE (MEDlars onLINE. International literature) and LILACS (Latin American and Caribbean Health Sciences). Unfortunately, most of this information has been published in Spanish and this pressumable idiomatic barrier could be a factor to explain why this information is not well disseminated and known amongst the non- Spanish speaking scientific communities.

One of these remarkable differences is the fact that in the industrialized world, *C. Coli* accounts for less than 5% of all *Campylobacter* infections. In South America *C. Coli* is isolated from about 25% of the diarrheal illness produced by *Campylobacter*. This species has also been isolated with high frequency from river water (26.5%) commercial chicken livers for human consumption (41.2%) in Chile and from sewage (25%) and chicken (19.8%) in Brazil thus, suggesting a possible environmental and food consumption linkage with this diarrheal agent high frequency.

Campylobacter species are rarely isolated from healthy individuals in developed countries. In contrast, a higher frequency of healthy carriers is a regularly observed epidemiological fact in developing countries. Perhaps, this could be related to environmental sanitary conditions which promote much higher transmission opportunities of *Campylobacter* from their natural sources to children at an earlier age, than it occurs in developed countries.

In general, the clinical symptoms and signs observed amongst patients from developed and developing countries do not greatly differ. However, in developing countries a high frequency of dehydration is often reported, which is probably due to the lower nutritional status diagnosed in most of the patients included in the clinical trials.

Another important aspect to point out is the fact that in developed countries, the diagnosis and search for *Campylobacter* is well established and ruled by the public health national systems with record, notification, epidemiological control systems and government regulations in relation to the presence of these bacteria in sources that could represent a health risk for the population, such as food of avian origin.

In Chile, and probably in other countries in South America, there is not such an organization nor regulations. In fact, in Chile the Sanitary Code and Regulation does not clearly states the specific search for *Campylobacter* in food; therefore, it is not compulsory to carry out such a search. On the other hand, the National Laboratories Network, ruled by the Chilean Public Health Institute

depending on the Health Ministry, does not include the diagnosis of *Campylobacter* in the recommended coproculture methodology either. Thus, the real magnitude of the epidemiological problem these bacteria represent is unknown. It is possible that a similar situation takes place in other countries in South America with an evident lack of government regulations. However, the information on *Campylobacter* originated in these countries, is the consequence of the personal interest of some researchers and Health Centers but not of a global public health policy such as the one designed for *Salmonella*.

In South America *C. jejuni* and *C. Coli* have been isolated from children with and without diarrhea, from wild and domestic mammals and birds, and from foods, sewage and river water. To illustrate this, Tables I and II show the isolation frequencies from each of these sources reported in some published works or obtained from personal communications of South American researchers.

In both, developed and developing countries, these microorganisms are widespread in the animal kingdom recognizing a great variety of animals as reservoir that could be source of infection for humans, other animals, foods and surface water bodies.

Although most *Campylobacter* infections are self-limiting and do not requiere antimicrobial therapy, in some clinical instances it is necessary the prescription of antibiotics. Usually, the decision to prescribe antimicrobial therapy is adopted when the patient presents one or more of the following signs: fever, bloody diarrhea, more than eight stools in 24 hours, worsening symptoms or persistent symptoms for more than one week. *Campylobacter* bacteremia, infection in an immuno-comprised host as well as infection during pregnancy are other clinical situations that require antibiotic therapy. The drugs of choise are erythrormycin and fluoroquinolones, especially ciprofloxacin. Cloramphenicol, tetracycline and gentamicyne have also been used. However, from the late 70´s and early 80´s the isolation of strains resistant to these antibiotics have been reported with a remarkable fast and increasing resistance to fluoroquinolones from the second half of the 80´s.

It appears to be that in Latin America *C. jejuni* and *C. Coli* were simultaneously isolated first in Costa Rica and Brazil in 1979, from cases of diarrhea and thereafter, studies have been carried out in several South American countries in order to know the susceptibility patterns of these bacteria to antimicrobial agents. Resistant strains to tetracycline and ampicillin were found in Brazil in 1983. A study carried out in Sao Paulo using the double dilution agar method (DDAM) reported that the resistance of *C. jejuni* and *C. Coli* to tetracycline had a bimodal distribution with a group of susceptible strains to very low concentrations (<1 mg/ml) and another to concentrations >32 mg/ml. In the case of *C. jejuni* isolated from humans, 66.9% of the strains were susceptible to <1 mg/ml and 19.2% were inhibited by tetracycline concentrations higher than 32 mg/ml whereas for *C. Coli* this distribution was 63.7 and 26.3%, respectively. In *C. jejuni* strains isolated from hens 45.3% were inhibited by <1mg/ml and 34.5% by more than 32 mg/ml of tetracycline. This bimodal distribution was also reported in developed countries as well as in some South American countries such as Chile, where in 1989 the 69% of *C. jejuni* strains isolated from hens were inhibited by less than 1mg/ml of tetracycline and 14% were resistant to more than 32 mg/ml. Ten years later, studying *C. jejuni* strains from the same origin, but using the E-test method, it was found that 15.1% of the strains were resistant to concentrations higher than 32 mg/ml of this antibiotic. Although the difference is not striking and we can speculate that no substantial resistance increase has taken place at least in Southern Chile, it is worth noting however, that in 1999 a 1.8% of the human isolates were resistant.

In other places in South America, the presence of tetracycline resistant strains has been determined by using the disc diffusion method (DDM). In a study carried out in Belo Horizonte (Brazil) in 1985, on a reduced number of *C. jejuni* strains isolated from acute diarrhea, it was found that 9.1% showed resistance to tetracycline. In Argentina, using the same method, it was found that 40.8% of the *C. jejuni* strains and 30.6% of the *C. Coli* isolated from diarrhea from a children hospital (Pediatric Hospital Dr. Garrahan) between 1997 and the first semester of this year, were resistant to this antimicrobial agent.

In relation to ampicillin, already in the first South American studies resistant strains to this antibiotic were found. Using DDAM, the frequency of resistant strains in Sao Paulo (Brazil) was 26.9% and 10.8% for *C. jejuni* isolated from children and hens, respectively. In human isolates of *C. Coli* this resistance was 15.8%. A study carried out in the central region of Chile (Santiago) using DDAM, showed that the frequency of ampicillin isolated resistant strains were 6.5%, 25% and 8.3% in human, poultry and porcine, respectively. In Southern Chile in 1989, strains isolated from hens showed 11% resistance to ampicillin for *C. jejuni* and 3% for *C. Coli* using the DDAM. In 1999, using the E-test method the percentage of canine strains of *C. jejuni* resistant to ampicillin was 9% and in poultry strains it was 22.6% being all of them beta lactamase producers. In our region, in strains isolated from hens the resistance to ampicillin has increased to 100% and in human strains it has been detected in 4.6% of the cases being always beta lactamase positive. In

Argentina, however, the resistance to ampicillin in strains isolated from children is higher since the detection using DDM showed 41% of *C. jejuni* and 47.2% of *C. Coli* resistant strains.

In relation to erythromycin, none of the studies carried out in South America in the 80´s showed the presence of resistant human derived *Campylobacter* strains to erythromycin except for one isolate of *C. jejuni* from Rosario, Argentina. At present in Buenos Aires, 3.1% of the *C. jejuni* strains and 6.3% of the *C. Coli* isolated from children are resistant to this antibiotic. In 1986 in Lima, Peru, 100% of the *C. jejuni* strains were susceptible to erythromycin. Today, however, 17% of them show resistance and 61% are inhibited by erythromycin concentrations ranging from 1 to 4 mg/ml. In 1985 in Santiago, Chile, no resistant *C. jejuni* strains were found in children with diarrhea, however, in the strains isolated from bovine, hens and pigs the frequency of resistant strains was 5.6, 8.3 and 13%, respectively. In Southern Chile no resistance to erythromycin was found in *C. jejuni* isolated from hens in 1989. Surprisingly however, in 1999 a high frequency (58.5%) of *C. jejuni* resistant strains to this drug was observed in hens fecal samples. For several years we have been doing a screening with DDM for erythromycin resistance in human strains and we have only found 7 resistant strains, 4 of which were isolated this year and 2 corresponded to *C. jejuni* and 2 to *C. Coli*, which in total represent 5.6% of the isolates carried out this year. Data from Peru, Argentina and Chile point out that the resistance of *Campylobacter* to erythromycin is an increasing phenomenon and it is likely to see in the future a higher frequency of clinical strains resistant to this antibiotic.

The finding that strike the most is the explosive appearance of resistant strains to fluoroquinolones in the last years. This phenomenon was reported initially in Europe in the second half of the 80´s following the introduction of this antimicrobial agents in the veterinary practice. Later, it was also reported in many other industrialised countries as well as in developing countries. In South America, the first trials appeared to be performed in Southern Chile in 1989 in *C. jejuni* and *C. Coli* strains isolated from hens where no resistance was observed. In another study, conducted in 1992, none of the *C. jejuni* and *C. Coli* strains isolted from children, hens, dogs, river water, aquatic birds and chicken livers were resistant to ciprofloxacin. Seven years later, however, in C jejuni isolated from hens in the same region, a 12.5% of resistant strains was found.

However, in 1995 during a survey of *Campylobacter* in fecal samples of pigs, dogs and cats, *Campylobacters* were isolated from 57 samples (32.2%), and 4 strains (3 *C. jejuni* and 1 *C. Coli*) were resistant to nalidixic acid (7.1%). All the nalidixic acid resistant strains showed cross resistance to ciprofloxacin, norfloxacin and enoxacin by the DDM and by the quantitative method of Wilkins and Thiel (MIC >40mg/ml). In the same region in 1999, 12.5% of the hens isolated *C. jejuni* strains were resistant to ciprofloxacin through the E-test. Recently, in the central region of Chile (Santiago) *C. jejuni* strains resistant to ciprofloxacin (50%) and norfloxacin have been also isolated. However, fluoroquinolones resistant strains have not yet been found in human beings at least in the South of Chile. In Peru, however, 78% of the *C. jejuni* isolated from clinical cases have been resistant to ciprofloxacin (DDAM). In the last three years in Buenos Aires (Argentina) 59.6% of *C. jejuni* and 49.1% of the *C. Coli* isolated from a children hospital (Pediatric Hospital Dr. Garrahan) were simultaneously resistant to both ciprofloxacin and norfloxacin. Parallel to this, in a study carried out in the city of La Plata near Buenos Aires, all the strains isolated from chicken livers were resistant to norfloxacin and, in addition, C. jeuni and *C. Coli* floroquinolone resistant strains were also isolated from porcine aborted fetuses and from the amniotic fluid from the mothers.

All the reports from South America show the fluoroquinolone resistance associated to nalidixic acid resistance. Data from the same geographic region show that fluoroquinolone resistance is a phenomenon also present and expanding in our countries. Probably, these data only represent the tip of the iceberg bearing in mind that in our countries there are not sanitary regulations in relation to the detection of *Campylobacter*, either in clinical samples as well as in animal-derived food, considered as risk factors in the transmission of these bacteria. Therefore, it is necessary to think that we are likely to face in the future a high resistance in strains from clinical cases.

Selection of resistant bacteria is strongly associated to the intensive use of antimicrobial drugs. In the case of the explosive emergence of fluoroquinolone- resistant *Campylobacter* strains, there is a common belief that this is due to the drug-bacteria contact favoured by their employment in veterinary and human medicine.

C. jejuni and *C. Coli* are zoonotic bacteria that recognize a great variety of animals as their natural reservoir. It is possible that poultry is the most important reservoir from the public health point of view, due to the fact that they are food-producing animals involved in the spread of *Campylobacters* to humans. On the other hand, *Campylobacter* species are the most frequent cause of bacterial food-borne illness in the United States and probably in other industrialized countries. There is also scientific evidence that the use of quinolones in food-producing animals promotes the

development of resistant strains that could infect humans through the food chain. In South America, despite the data showing the existence of fluoroquinolones resistant *Campylobacter* in poultry, the lack of epidemiological control systems and government regulations, in relation to the presence of these bacteria in food, prevents us from reporting data on this respect, thus leaving here an epidemiological gap that should be reverted in the future in order to design the appropiate control measurements.

Paralell with the expansion of the aquaculture industry, the use of antibiotics in fish farming has increased and the development of antibiotic resistance in fish pathogens, such as Aeromonas hydrophila and Vibrio salmonicida, has been reported. In fish farming the quinolones oxolinic acid and flumequine are extensively used to treat various fish infections. If the use of quinolones in warm blood food-producer animals is a risk for the public health, their use in fisheries could also be considered as such, since these drugs are not easily broken down and can be demonstrated in the environment months after they have been used at fish farms. As resistant strains in pathogenic bacteria have been demonstrated in fish, there is also the risk that the same could happen in bacteria of medical importance associated with marine activity, such as some species of *Campylobacter* that can be isolated from both aquatic birds and shellfish.

The use of fluoroquinolones in the treatment of human campylobactriosis was found to be a risk factor for resistant infections since it has been observed that *C. jejuni*, for example, is prone to develop resistance during treatment with these drugs. It is possible that in developing countries there are more possibilities to induce resistance to quinolones in *Campylobacter* due to the use of these drugs for the empiric treatment of not only the gastroenteritis but also other infections such as the urinary tract infection. If we add to the above-mentioned the self-medication, and the high rate of intestinal *Campylobacter* carriers, the possibility to generate resistant strains through this route would have great importance.

The acquisition of resistance to antibiotic by these bacteria seems not to involve changes in their pathogenic capacity. Under these circumstances however, the infection will be more difficult to eliminate. On the other hand, the consequence of the treatment failure is a more prolonged diarrhea which in turns, brings about economical consequences, since it involves the use of alternative drugs and the loss of productivity due to the days off work. In addition, the treatment failure also involves the spread of resistant strains to the environment and to the community. Moreover, to have a more prolonged diarrhea due to the treatment failure, could increase the risk of the onset of haemolytic uremic syndrome or Guillain-Barré syndrom as complications of the diarrhea. It is equally necessary to consider in the infection by a resistant strain, the possibility that the diarrhea complicates with a protein-loss enteropathy or that it interferes in the optimal weight gain, especially in children from developing countries.

It is worth pointing out that the *Campylobacter* infections in immunocompromised patients or in patients underlying health problems are usually severe and sometimes with bacteremic complication. Under these circumstances, the treatment failure could be fatal if the infection is produced by a resistant *Campylobacter* strain.

In nalidixic acid-rsistant clinical and animal isolates it has been found cross-resistance with fluoroquinolones. Having in mind the increasing isolation of nalidixic-acid resistant *Campylobacters*, the importance of testing the susceptibility of *Campylobacter* strains for identification purposes, has lost its original meaning. However, susceptibility to nalidixic acid could be acting as a sentinel giving valuable information concerning the susceptibility of the isolates to fluoroquinolones. Almost in all the studies carried out in developed and developing countries, none of the nalidixic-acid susceptible strains were resistant to fluoroquinolones.

It is clear that the food-borne pathogens, *C. jejuni* and *C. Coli*, have become increasingly resistant to antibiotics changing clinical, epidemiological and laboratory diagnostic aspects of *Campylobacter* infection. However, other pathogenic species of *Campylobacter*, such as C. fetus ssp. fetus, could also develop antibiotic resistance. The latter was reported in human immunodeficiency virus-infected patients treated with ciprofloxacin. If antibiotic resistance can be acquired by enteropathogenic and opportunistic species of *Campylobacter* as a consequence of the pressure, due to the use of drugs, then, the question is: Could the same happen with the emergent species of *Campylobacter* or with the related species Arcobacter burtzleri, which can also produce infection in humans? Arcobacter butzleri was isolated this year in Southern Chile from commercial liver chicken (40.9%), river water (83.3%), shellfish (40.8%) and from a case of chronic diarrhea in a child.

Abroad travelling of citizens from developed countries is considered a risk factor for acquiring quinolone-resistant *Campylobacter* infections. However, tourists and soldiers deployed overseas can also acquire infections by resistant strains to other antibiotics, especially in developing countries where *Campylobacters* are endemics. In addition, the food trade of potentially contaminated food with resistant strains, such as poultry meat, should

also be considered as a risk factor for the spread of the antibiotic-resistant *Campylobacter* strains, unless a good sanitary control system is available.

Considering that both activities -tourism and food trade- are of economical concern, and that the spread of resistant strains could result in an increase of the morbility with the aggravating fact that these infections also have economical consequences for the patient´s family and country; health authorities from developing countries should make the necessary arrangements to minimise the problem.

We mentioned at the beginning that there are not epidemiological control systems and government regulations in relation to the presence of these bacteria in sources such as poultry food, that could represent a health risk for the population.

These measurements should be implemented in the short term together with the incorporation of the *Campylobacter* diagnosis as a routine in coprocultures. This will bring along the need to implement educational and training programmes for the health staff either by incorporating them to the study programmes at the universities or by giving training courses. In addition, surveillance systems with standard methods, comparable amongst different laboratories and countries, should be implemented. This surveillance systems should include samples from all relevant sources of public health concern such as the reservoirs, food and human beings.

Probably the conduction of collaborative studies amongst scientists of developed and developing countries, the collaboration of scientific societies and international organizations and educational and training programmes, could be elements to contribute to the establishment of control and diagnosis programmes in the developing countries.

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Table 1. Isolation rates (%) of C.jejun*i and C. Coli from diarrheic and normal children in some countries of South America*

Country	C. jejuni		C. coli	
	Diarrhea	Control	Diarrhea	Control
Argentina	5.6	NS	0.6 [7.3]	NS
Bolivia	4.4	NS	7.3 [45.4]	NS
Brazil	5.8	4.9	2.2 [37.9]	2.0
Chile - Santiago	9.2	4.0		
Chile – Valdivia	14.1	4.0	5.4 [27.7]	3.6
Colombia	14.4	3.7	2.4 [14.3]	1.2
Ecuador	23.0*	NS		
Panama	11.7	NS		
Peru	15 - 23	NS		
Venezuela	13.0*	9.0*		

* Referred to as C.jejuni/coli or *Campylobacter* spp.
NS Not studied
[ ] frequency of *C. Coli* in relation to all the cases of *Campylobacter* diarrhea

Table II. Isolation rates (%) of C. jejuni and C. Coli from animals, food, water and sewage in some countries of South America

Country	C. jejuni/coli	C. jejuni	C. coli
Argentina			
cattle	1.7		
swine	5.9		
chicken livers		35.2	1.6
beef	3.2		
Brazil			
dogs		35.2	7.6
cats		23.8	7.9
cattle		53.3	8.4
chickens		66.9	19.8
chicken meat	78.7		
pork meat	35.0		
sewage		56.2	25.0
Chile			
dogs		34.7	16.7
cattle		22.5	7.5
swine		15.0	55.0
chickens		45.0	15.0
ducks		66.0	7.0
geese		28.4	18.3
sparrows		28.0	12.0
aquatic birds		11.8	11.8
chicken livers		21.7	69.6
river water		9.5	24.3
Colombia			
milk		4.0	1.0
Peru			
dogs		25.0	
cats		21.0	
ducks		18.2	
chicken		61.4	
domestic monkeys		10.6	21.3
wild monkeys		4.6	16.3

11. What can be learned from surveillance and register studies?

Kåre Mølbak,

In Denmark, the incidence of gastrointestinal infections due to *Salmonella, Campylobacter* and *Yersinia* has showed marked changes over the last 20 years (Figure 1). The incidence of *Salmonella* infections increased in the late 1980s primarily due to *S.* Typhimurium infections from broiler chickens. The "second *Salmonella* wave" peaked in 1993; the primary source being pork meat. Finally, in the late 1990s, shell eggs contaminated with *S.* Enteritidis became a major source of *Salmonella*-infections, and this "third *Salmonella* wave" peaked in 1997. Over the same period, various *Salmonella* control programs were implemented. These programs, with an emphasis on the pre-harvest control of *Salmonella* in broilers, layers and swine, have markedly reduced the *Salmonella* levels in poultry flocks and in swine herds by testing and intervening at the herd level. Furthermore, these interventions have had a major impact on the incidence of human *Salmonella* infections. However, the incidence of *Campylobacter* started to increase in 1992, and during the last 8 years the number of cases have increased from 1,129 in 1992 (22 per 100,000 inhabitants) to 4,380 in 2000 (83 per 100,000; preliminary figures). This almost exponential increase has been observed in several other countries as well (1). By contrast to the *Salmonella* situation, the *Campylobacter* increase has not been paralleled with similar changes in the *Campylobacter* levels in animals or food from animals, and no control plans have been implemented.

Surveillance data, as showed in Figure 1, are traditionally used primarily for graphing trends and identifying outbreaks. In the present paper, I will give some examples of how these data are used as the backbone for studies to address the epidemiology and health impact of *Campylobacter* infections. My examples include:

- Mortality up to two years after *Campylobacter* infection as well as the associated co-morbidity
- Seasonality of *Campylobacter*
- A detailed analysis of the *Campylobacter* incidence to address the recent increase

The data presented in this report should be regarded as preliminary.

Mortality associated with *Campylobacter* infections

Infections with gastrointestinal bacterial pathogens are recorded in the Danish Registry of Gastrointestinal Infections. Most of the notifications are individual laboratory reports accompanied by the so-called CPR number. The CPR number is a unique identifier of all Danish citizens, and demographic data including survival is continuously updated by the Danish Civil Registry System on the basis of the CPR number. To determine the acute and long-term mortality (i.e., up to two years after infection), we linked the individual reports in the Registry of Gastrointestinal Infections with the Civil Registry System (CRS). Furthermore, to examine the role of co-morbidity, data on hospital discharge diagnosis were obtained from the National Discharge Registry, which is another national registry based on the CPR numbers. To determine the survival of non-exposed individuals (i.e., persons without a recognized *Campylobacter* infection), we randomly selected 10 persons from the CRS per case – matched for age, sex and county. This enabled us to compare the survival of the patients with the survival of the general population.

The analysis included a total of 16,179 *Campylobacter* cases, registered from 1991 to 1999. Table 1 shows the mortality risk after 30 days. After the subtraction of the background mortality we estimated a case-fatality rate of 4 per 1000; the rate being highest in elderly patients. To address the mortality at a two years follow-up, the data were analyzed by conditional Cox proportional

hazards regression. Overall, patients with *Campylobacter* infections had a 1.9 times excess mortality (95% confidence interval 1.7 to 2.2); $p < 0.0001$ compared with the unexposed controls.

The data were further linked to the National Discharge Registry. A number of diagnoses were found to be to be more common in *Campylobacter* patients than in unexposed individuals. This included HIV/AIDS, hematologic and solid malignancies, diabetes, gastrointestinal diseases and allergic disorders (which may be associated with the use of corticosteroids) and other chronic disease. Several of these diagnoses were associated with an excess mortality. By fitting a model which took this mortality into account, we were able to determine the proportion of the deaths which could be attributed to the different diagnostic groups. Table 3 shows that *Campylobacter* infection itself could be associated with 26% of the deaths. HIV infection, malignant diseases and asthma were the most important co-morbidity diagnoses with an effect on mortality.

The present study may be regarded as a study of *Campylobacter* mortality based on a large and unbiased sample of patients from an industrialized country with an advanced and free health care system. Most episodes of Campylobacteriosis were self-limiting with a very good prognosis *quo ad vitam.* Nevertheless, we found a significant acute and long term excess mortality of the patients compared with unexposed individuals. The acute phase mortality, about 4 per 1000, may be higher than previously thought. The two-years excess mortality was partly associated with HIV infection and other chronic diseases, but approximately 26% of the excess deaths were attributable to *Campylobacter* itself. Due to the high incidence of *Campylobacter* infection, this excess mortality may be of public health importance. The interaction between HIV infection and *Campylobacter* was reflected by a 5.8 times excess mortality of patients in the age group 30-39 years. It is very likely that Campylobacteriosis is particular severe in HIV-positives and other immunosuppressed individuals – an observation which may be important also for developing countries where both HIV and *Campylobacters* are highly endemic.

Seasonality of *Campylobacter*

Countries in temperate zones have a seasonal distribution with an increase in the spring and a well-defined summer peak (1). In Denmark the usual peak occurs at week 31 to 33, with a remarkable constant pattern year after year. Figure 2 shows that surveillance in broiler flocks shows a similar seasonality. Some years the percent positive broiler flocks may peak a little earlier than humans, but overall, the seasonal pattern of contamination of broilers and patients is similar. This may indicate that broilers are the primary source of *Campylobacter* infections, or that humans and broilers are becoming infected from the same source. Some studies have reported mismatches between the seasonal distribution in poultry and humans. For example, in Norway, the proportion of colonized flocks peaked in the autumn, i.e., after the peak in human cases (2), and in the UK no apparent season variation was found in a one-year study of *Campylobacter* presence in 49 broiler flocks at slaughter (3).

To address the issue of seasonality in a systematic way, Nylén, Palmer and coworkers analyzed the distribution of *Campylobacter* infections in humans (4). The analysis was primarily based on surveillance data from Denmark, Finland, New Zealand, Scotland, Sweden, and Wales. In the Nordic countries, the peak occurred between week 29 and 35, whereas the peak appeared earlier in Wales (week 23 to 27) and Scotland (week 24 to 27). Furthermore, the seasonality appears to be more pronounced by increasing latitude.

Across different regions of the world, a better understanding of the seasonal distribution of *Campylobacter* infections and the relations to the occurrence of the bacteria in different reservoirs are warranted. Such data, presented and analyzed by standard methods, may be helpful to obtain a clearer picture of the dynamics of *Campylobacter* transmission, and to challenge the hypothesis that poultry is a major source of infection.

Campylobacter infections in Denmark, 1992 to 1999

Epidemiological studies have suggested that the sources of sporadic *Campylobacter* infections are likely to be multiple, with poultry as a principal one. However, the recent emergence of *Campylobacter* infections in many countries is not well explained. The emergence may not affect all population groups at equal rates, and a better understanding of the demography of Campylobacteriosis as well as temporal and spatial trends could give clues to a hypothesis regarding the recent increase. In the present analysis, routine laboratory surveillance data from seven Danish counties were analyzed to assess effect modification between demographic risk factors and the temporal trend.

During the period 1992 to 1999, the annual number of *Campylobacter* infections in seven counties where Statens Serum Institut is in charge of the primary diagnosis increased 3.5 times from 475 in 1992 to 1,676 in 1999. Figure 3 shows the increase expressed as relative rates adjusted for the

effects of age, gender, season, and county. The increase in the relative rate for domestic cases followed a log linear pattern, with an estimate of 2.96 for 1999 compared with 1992. The temporal trends were modeled under the assumption of a linear trend in the annual increase. Table 4 shows the main model. For the non-travel associated cases, the annual increase in *Campylobacter* infections was estimated to 1.14 ($p < 0.0001$). There were also significant effects of age, gender, season, and county (all p-values < 0.001). The incidence was highest in infants and children below 5 years, but there was a second peak in the age group 15 to 24 years. Females (RR 0.93) had lower rates than males, mainly due to a lower *Campylobacter* incidence in girls below five years of age. Furthermore, there was a marked seasonal variation with high incidence in July to September (RR 1.54) and low incidence in January to March (RR 0.72), using October to December as reference.

For travel associated cases, there was no linear trend in the annual increase, and there was no significant difference between males and females. The effects of age, season and county exhibited largely similar patterns as the domestic cases (Table 4).

The following analyses examined effect modification by age, gender, county and season. There were significant interaction between the annual increase in *Campylobacter* and age, county and season (all p-values < 0.001), suggesting that the incidence did not increase by an equal rate according to the demographic factors and season. By contrast, the annual increase in males and females were similar ($p = 0.69$). Table 5 shows the annual increase by season and age-group. The annual increase was statistically significant throughout the year but was highest during the period April to June (annual increase 1.20) and lowest October to December (1.09). The incidence of *Campylobacter* in infants did not increase significantly in the period (annual increase 1.01), but was high in adults 45 to 64 years (annual increase 1.19), closely followed by the ages 25 to 44 (annual increase 1.18) and 15 to 24 years (annual increase 1.17).

The “excess increase” during the summers and in the mid-aged population shows on the crude numbers. In 1992, 56% of the patients were found in the months April to September, whereas 68% were found in these months in 1999 ($p < 0.001$), and in 1992, children under 5 years accounted for 24% of the cases, a proportion which declined to 14% in 1999. By contrast, 34% of the patients were 25 to 64 years of age in 1992, a proportion which increased to 51% in 1999.

Consumption of poultry and poultry products have in almost every case-control study been identified as a risk factor for sporadic *Campylobacter* infections. This include the recent Danish study, conducted from 1996 to 1997 (5). Whereas the level of contamination of poultry products may have been more or less stable in the period, the overall consumption has increased from 1992 to 1999 by 50% (4). The consumption of poultry in Denmark was largely constant until 1992. In addition, there has been a shift from frozen to fresh poultry and a larger variety of cuts and preparations which require more handling at the processing level (pers. comm., Danish Board of Poultry Producers). Hence, an increase in the consumption of poultry may be put forward as an hypothesis which is in line with the present analysis, and warrants further examination.

Meat eaten at a barbecue has also been found as a risk factor for sporadic *Campylobacter* infections both in Denmark and elsewhere. The effect modification by age and season corroborate the notion that barbecuing may be one of the causes of the increase in *Campylobacter* infections. There are indications to suggest that home barbecues in Scandinavia has become increasingly popular over time, but again more data are warranted to examine this hypotheses. Finally, other changing habits in diet and cooking practices and experience may be of importance and are not refuted by the present analyses.

Conclusions

The present paper suggests that surveillance has the potential of addressing new and important aspects of the health significance and epidemiology of *Campylobacter* infections. Similar studies from other countries are needed, and therefore national and international surveillance systems should be improved and designed to be filed in a ”one line per patient” form. Whenever possible, demographic details and information on foreign travel should be included, and the data on incidence should be evaluated by separating domestic versus travel associated cases. To analyze the recent increase observed in several countries, interactions with age, geography, season and if possibly other factors need to be addressed. It is likely that the increase does not affect all population groups at an equal rate, and an understanding of any particular trends in special groups may shed light on the increase.

The seasonality of *Campylobacter* infections across regions and countries should be further evaluated. These analyses should address the week of maximum and minimum number of cases as well as the magnitude of the oscillation and temporal and spatial trends. If possible, the seasonal distribution of human cases should be compared with the findings in different reservoirs, including poultry.

Surveillance data has the potential of forming the backbone of registry based studies. These may - with limitations - be used for addressing different aspects such as mortality, other major disease outcomes, and the health impact of antimicrobial resistance. Similar data are warranted for Guillian-Barré syndrome.

Acknowledgments

I thank Morten Helms, Pernille Vastrup, Peter Gerner-Smidt, and Jørgen Engberg at Statens Serum Institut; Jakob Neimann, Flemming Bager and Henrik Wegener at the Danish Zoonosis Centre; Gunnar Nylén and Steven Palmer, University of Wales College of Medicine. The Danish Research Centre for Environmental Health supported the mortality study.

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Table 1. Case-fatality rate (30 days mortality) of Campylobacter *infections, Denmark, 1991 to 1999*

Age-group	Number of Deaths*	Cases	Mortality risk (%)	Risk difference**
< 5 yrs	1	2,737	0.04%	0.03%
5 to 54	3	11,556	0.03%	0.02%
55+	40	1,886	2.12%	1.76%
All	44	16,179	0.47%	0.38%

* at 30 days after receipt of specimen in laboratory
** the mortality risk after subtraction of the population mortality risk in the 30 days time window

Table 2. Two-years mortality after Campylobacter *infection. A population based registry study including 16,179 patients and 10 unexposed individuals for each patient. Mortality was determined by conditional proportional hazards regression, and all mortality ratio expressed as relative to the unexposed population. Denmark, 1991 to 99.*

Age group (years)	Mortality ratio
0-29	1.7
30-39	5.8
40-49	2.8
50-59	1.6
60-69	1.5
70-79	2.3
80+	1.5
Overall	1.9

Table 3. Two-years mortality after Campylobacter *infection. The table shows the estimated population attributable proportion (etiologic fraction) of the excess mortality pertaining the* Campylobacter *infection itself and associated co-morbidity. Denmark, 1991 to 99.*

Estimated population attributable proportion to Campylobacter *excess mortality:*

Campylobacter	26%
HIV-infection/AIDS	18%
Asthma	4%
Solid malignancy	2%
Lymphomas	2%
Diabetes	1%
Liver disease	1%

Table 4. Temporal and demographic determinants for the incidence of Campylobacter*-infections, seven counties of Denmark, 1992 to 1999. The table shows relative risks obtained from a multivariate Poisson regression model.*

Determinant	Domestically acquired (N= 7459) RR (95% CI)**	Travel associated (N=1343) RR (95% CI)**
Yearly increase since 1992	1.14 (1.13-1.16)	1.00 (1.00-1.00)
Quarter of year		
Jan-Mar	0.72 (0.67-0.78)	0.87 (0.73-1.04)
Apr-Jun	1.00 (0.94-1.08)	1.04 (0.88-1.23)
Jul-Sep	1.54 (1.45-1.63)	1.34 (1.16-1.55)
Oct-Dec	1.00 (reference)	1.00 (reference)
Age group (years)		
<1	6.23 (5.23-7.42)	8.91 (4.60-17.26)
1-4	4.47 (4.04-4.95)	2.56 (1.75-3.74)
5-14	1.37 (1.23-1.53)	1.00 (0.69-1.45)
15-24	2.34 (2.12-2.58)	2.05 (1.51-2.78)
25-44	1.46 (1.33-1.60)	0.86 (0.63-1.16)
45-64	0.90 (0.81-1.00)	0.72 (0.53-0.98)
65+	1.00 (reference)	1.00 (reference)
Gender		
Female	0.93 (0.89-0.97)	1.08 (0.97-1.20)
Male	1.00 (reference)	1.00 (reference)
County		
Frederiksborg	1.20 (1.11-1.29)	1.19 (1.01-1.40)
Roskilde	1.30 (1.19-1.41)	1.50 (1.23-1.83)
Storstrøms	1.19 (1.09-1.29)	1.35 (1.08-1.69)
Bornholm	3.07 (2.61-3.61)	6.30 (3.24 12.23)
Fyn	1.16 (1.08-1.24)	1.06 (0.91-1.23)
Ribe	1.71 (1.59-1.84)	1.66 (1.38.2.01)
Aarhus	1.00 (reference)	1.00 (reference)

* cases per 100,000 per year during the period 1992-99
** relative rate and 95 percent confidence interval

Table 5. Yearly increase in incidence of Campylobacter*-infections according to quarter of year and age of patients. Relative rates obtained from a multivariate Poisson regression models with interaction terms between calendar time and quarter of year respectively age group. Domestically acquired cases in seven Danish counties, 1992-99.*

Yearly increase 1992-99	RR (95% CI)*
Quarter of year	
Jan-Mar	1.10 (1.07-1.33)
Apr-Jun	1.20 (1.17-1.22)
Jul-Sep	1.16 (1.14-1.18)
Oct-Dec	1.09 (1.07-1.12)
Age group (years)	
<1	1.01 (0.95-1.09)
1-4	1.07 (1.05-1.10)
5-14	1.11 (1.07-1.15)
15-24	1.17 (1.14-1.20)
25-44	1.18 (1.16-1.21)
45-64	1.19 (1.15-1.22)
65+	1.09 (1.05-1.13)

* relative rate and 95 percent confidence interval

Figure 1. Number of registered cases with zoonotic Salmonella, Campylobacter *spp. and* Yersinia enterocolitica *in Denmark, 1980 to 2000. Denmark has a population of approx. 5.3 millions*

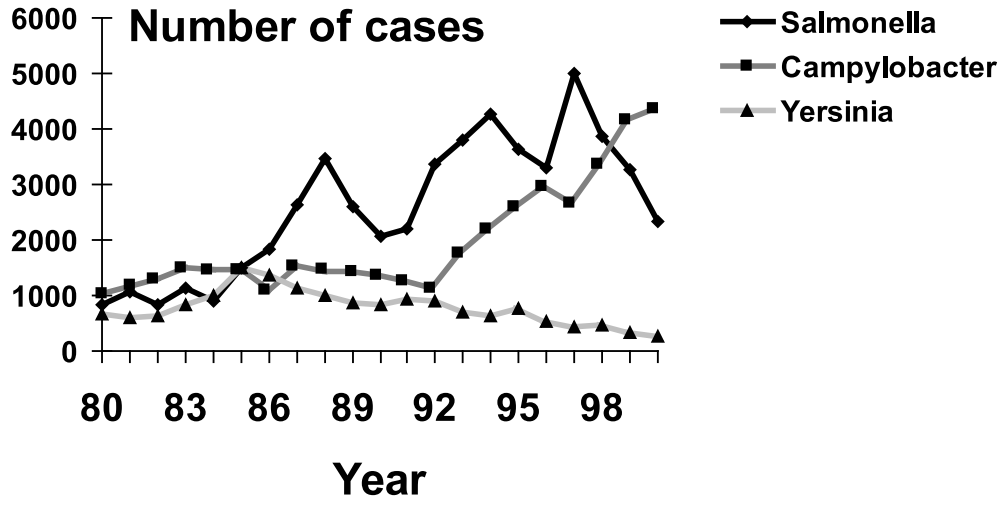


Figure 2. Number of human Campylobacter *infections and prevalence of* Campylobacter *infections in broiler flocks at slaughter by month, Denmark, 1998 to 1999.*

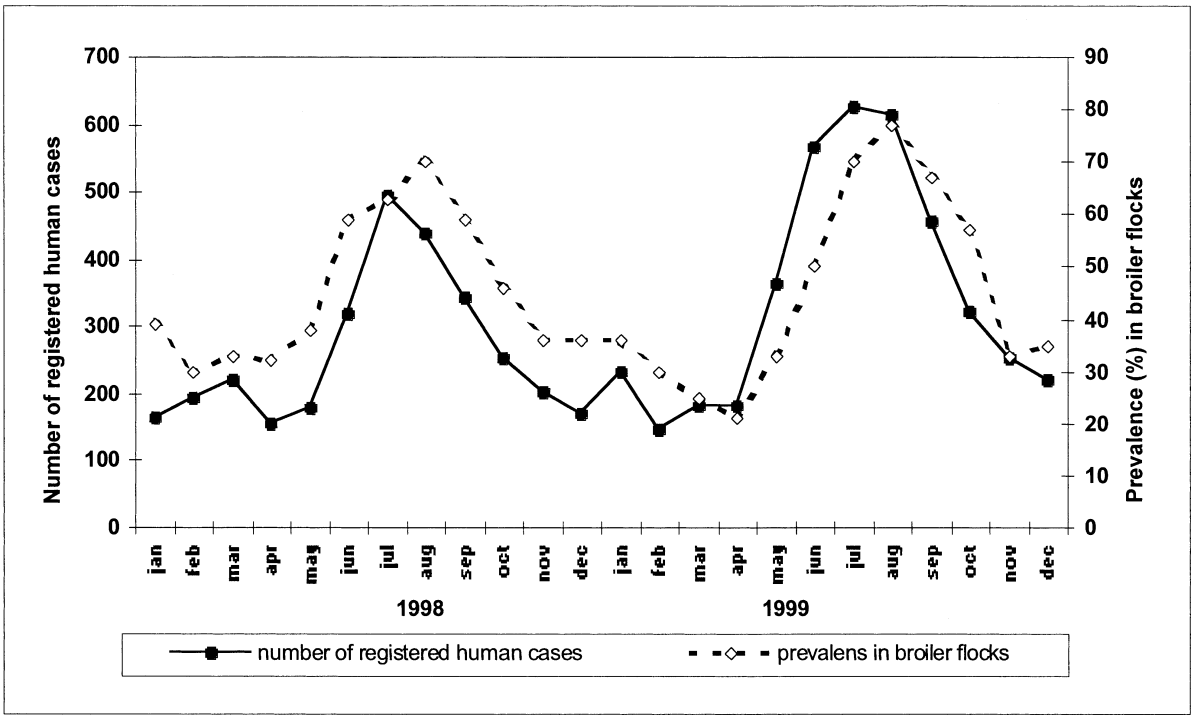
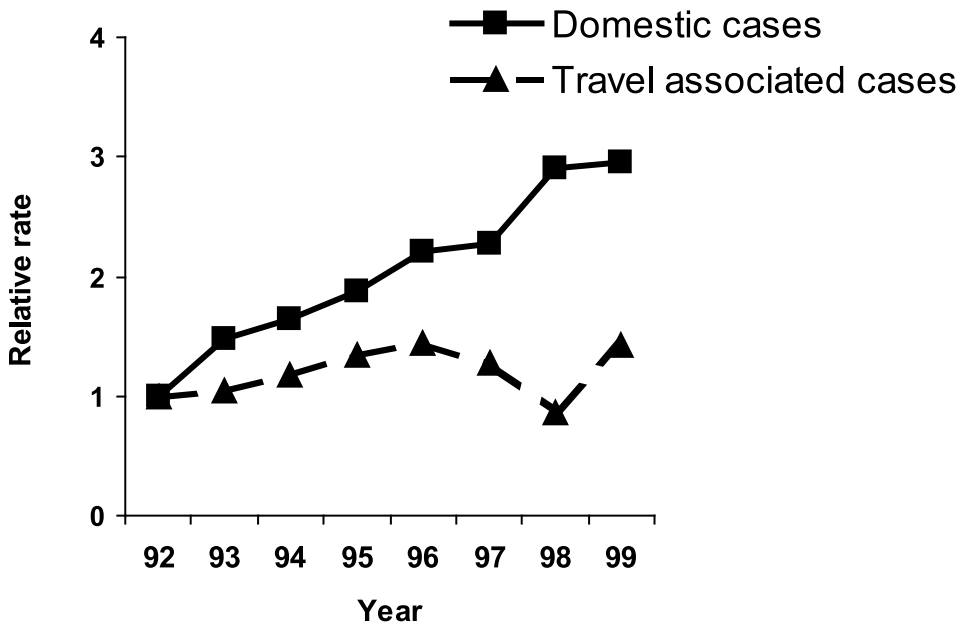


Figure 3. Campylobacter *infections in seven counties of Denmark, 1992 to 1999. The figure shows Poisson regression multivariate relative rates, with 1992 as baseline. The estimates are adjusted for effects of age, gender, county and quarter of year.*



12. Combining typing data and epidemiological information in *Campylobacter* surveillance – new opportunities

Jennifer A. Frost
Iain A. Gillespie
Sarah J. O'Brien

1. Introduction

1.1. Incidence in England and Wales

Campylobacter has emerged as the most commonly recognised bacterial cause of gastrointestinal infection in England and Wales since 1981 and the incidence of infection reached a peak of almost 60,000 cases in 1998 (www.phls.co.uk). The true number of cases is likely to be much higher. It has been estimated that, for every case of *Campylobacter* infection reported to national surveillance in England, there are a further seven in the community (Wheeler *et al*, 1999). This would indicate approximately 420, 000 *Campylobacter* cases per annum.

1.2. Outbreaks

Much of our understanding of the epidemiology of other enteric bacterial pathogens, such as *Salmonella*s and *E.coli* O157 is based on outbreak investigations. For *Campylobacters*, reports of outbreaks are few and far between. Between 1989 and 1999 there have only been 81 general outbreaks of *Campylobacter* infection reported to the Public Health Laboratory Service Communicable Disease Surveillance Centre (CDSC) compared with 100 or more *Salmonella* outbreaks most years.

1.3. Reference typing

Despite being such a common cause of infective gastro-enteritis, the epidemiology of *Campylobacter* infection is still poorly understood and until 1995 there was no national reference centre in England and Wales analogous to that which has existed for *Salmonella* since 1946. The PHLS *Campylobacter* Reference Unit (CRU) typing methods were piloted in Wales between 1996 and 1997. Between April 1997 and March 2000 the CRU provided a full reference service for two regions, with support for outbreak investigations throughout England and Wales. This generated typing data for approximately 16% of the infections reported nationally to CDSC. Both regions, North West England and Wales, used the reference data in tandem with some local enhanced surveillance to shed light on the relative contribution of the many potential sources of *Campylobacter* to human disease.

1.4. National *Campylobacter* surveillance

It was apparent that the benefits of having detailed strain characterisation could only be fully realised if these data were matched with comprehensive epidemiological data. Given that there are likely to be regional variations in risk factors, or at least regional variations in the relative contributions of a number of risk factors in causing human illness, a scheme which would be more representative of the national situation was required.
The regional system has now therefore been replaced with a sentinel system covering selected Health Authorities throughout England and Wales, ensuring that all NHS Regions are incorporated into the scheme.

1.5. Further details of the current national *Campylobacter* situation are given in the accompanying report for England and Wales (to be tabled at the consultation meeting).

2. The *Campylobacter* sentinel surveillance system

2.1. Aims

The aim is to generate hypotheses for human *Campylobacter* infection in a structured, systematic way using standardised epidemiological and microbiological information from a representative sample of cases in England and Wales. The evaluation of typing data, which is possible using this structured approach, includes data from outbreak investigations throughout the whole of the United Kingdom. A standard risk factor questionnaire is used which may be supplemented with additional data items for local analysis. The system is designed to provide robust population denominators and provide a statistically valid sample from which extrapolation of trends is legitimate. The sentinel system will also provide a focus for detailed studies on specific food, environmental and socio-economic issues impacting on *Campylobacter* epidemiology.

2.2. Participation and data collection

The sentinel system is a collaborative effort involving a wide range of public health professionals in the collection and dissemination of information regarding *Campylobacter* epidemiology in England and Wales and covers a population of just over 12 million people. Working arrangements between the District Health Authority (DHA) and Local Authority (LA) have been developed locally to ensure that relevant epidemiological data are collected on as many patients as possible. Laboratories send all *Campylobacter* isolates to the reference laboratory for typing while the questionnaire is sent to CDSC by the Consultant in Communicable Disease Control (CCDC) or Environmental Health Officer (EHO) according to local arrangements. Epidemiological and microbiological data are collated and analysed at CRU and CDSC. Reference laboratory reports are sent to the sending laboratory on completion of testing and a line listing for their district is sent to each CCDC monthly. Full data analyses are circulated quarterly. The first eight months are intended to be a pilot study to build data handling systems and clarify arrangements between participating laboratories and DHAs. The project will then run for two complete calendar years to enable valid analysis of both seasonal variation in subtype distribution and association between risk factors and subtypes.

3. Preliminary analysis of first three months data

3.1. May to July 2000

During the first three months of operation a total of 4,971 isolates of *Campylobacter* were referred to CRU from laboratories in fifteen district health authorities; 2,046 questionnaires were returned from affected patients. So far 1,081 combined epidemiological and microbiological datasets have been obtained. The disparity between total isolates referred and the number of questionnaires received occurs because laboratory catchment areas and DHA boundaries are not co-terminus, the former often serving more than one district. Short term data matching is subject to variations between DHAs in the time which elapses between submission of the isolate to CRU and forwarding of completed questionnaires to CDSC for analysis. Final analyses based on a full year's data will be more complete.

3.2. Patient data [all respondents]

Patients' ages ranged from less than one month to 99 years (Figure 1) with a small excess of males (52%) to females (48%). The duration of illness ranged from 1 to 125 days, a total of 20,744 days illness with an average value of 11 days. There were in total 11,678 days when patients were unable to work or undertake normal activities, an average of 7 days per patient. As might be expected, 96% of patients reported having diarrhoea of which a third reported bloody diarrhoea. While 86% of patients reported abdominal pain and 80% fever, only 34% reported vomiting. Importantly, 10% of cases were admitted to hospital for an average of five days, with a maximum stay of 30 days for one patient.

3.3. Microbiology [all referrals]

Predictably, 94% of the 4,971 *Campylobacter* isolates referred to CRU were *C. jejuni* with the majority of the remainder being *C. Coli*; there were eight *C. lari* and one *C. fetus*. There was a wide range of sub-specific variation; 57 serotypes and 69 different phage types were identified. Together these gave 612 different combined subtypes(Table 1). Only 73 isolates (1.4%) were untypable by both subtyping methods used.

The most common *C. jejuni* subtypes were sero-untypable PT1 (15% of isolates), serotype HS50 PT5 (3.5%) and HS50 PT34 (3.1%). Fifteen subtypes each accounted for more than 1% of the total *C.jejuni* isolates.

Among the matched laboratory and questionaire data the most common subtypes were serotype HS 8 phage type PT 1 and HS50 PT 1 each with 12 isolates. These ranked 14th and 5th overall.

Serotype HS8 PT1 did not feature in the top 20 *C. jejuni* subtypes in CRU data from Wales and North West England between 1997 and March 2000. Furthermore this subtype did not show any distinct seasonality in previous years. Of the 51 isolates of this subtype in this data series, 38 were isolated during May. Of these, 16 were isolated in the Northwest of England including eight of those for whom questionnaires have been received. Further local investigations failed to identify a common source for these infections.

3.4. Epidemiology [matched data]

Exposures to *Campylobacter*-associated risk factors in the two weeks prior to onset of infection were analysed in respect of travel away from home, contact with animals and a wide range of food and water exposures (Table 2).

3.4.1. Travel

This preliminary dataset was collected over the summer period and a total of 297 [19%] of patients reported having travelled outside the UK. The destinations visited reflected the wide range of countries visited by travellers from UK. A further 251 [16%] patients travelled within the UK, predominantly to rural and coastal areas.

3.4.2. Food and water

Seven per cent of patients reported having consumed unpasteurised milk in the two weeks prior to onset of illness, despite the fact that it is not widely available in the UK. A further 2% of patients reported consuming milk from doorstep deliveries where the bottle top had been pecked by birds.

Exposure to potentially contaminated water also featured strongly among the positive responses. Seven percent of patients had private as opposed to mains water supplies and an additional 7% reported having had water supply or engineering problems in the exposure period. Two per cent of patients had been exposed to river or stream water.

3.4.3. Animal contact

Both domestic and animal contact are recognised risk factors for *Campylobacter* infection and 58% of patients reported contact with animals, 8% of these had recently visited a farm.

3.4.4. Outbreaks and clusters

Although the time scale of this reporting system is such that outbreak detection is not a primary aim, it does have the potential for retrospective outbreak identification. Among the sporadic infections in this dataset, 10% of patients were aware of others with similar illnesses outside of their immediate household while illness among household contacts was reported by 16% of patients. This suggests that there are both household and general *Campylobacter* outbreaks which are not being reported.

3.5. Geographical data analysis

The collection of patient post code data enables the use of Geographical Information Systems (GIS) for data analysis. The monthly data listing reported to each Health Authority includes a map of the cases from their area together with specific maps for subtypes with suspected local clusters. As the database grows these data will be a powerful tool for analysis of socio-economic and climatalogical factors associated with *Campylobacter* infection.

4. Observations on preliminary data analysis

4.1. The sentinel system is a collaborative effort involving a number of agencies – the Public Health Laboratory Service , National Health Service Trusts, Consultants in Communicable Disease Control at District Health Authorities and Local Environmental Health Departments. As none of the above agencies have co-terminal catchment areas, data matching for the two components of the system, microbiological and epidemiological, has proved to be complex. The importance of good patient identifiers including the patient's home post codes cannot be over emphasised.

4.2. The time lapse between the laboratory identifying a *Campylobacter*, notifying the CCDC, and the patient receiving the questionnaire will be a mimimum of four days from the patient seeking medical advice and up to three weeks from exposure to the vehicle of infection. This makes patient recall bias an important factor in any analysis. This bias can be addressed by use of analytical tools such as the case-case study described later.

4.3. The time lapse between patient exposure and data analysis means that this surveillance system will be unlikely to result in any immediate interventions. However the matching of laboratory and patient derived epidemiological data on a national and local basis will faciliate hypothesis generation which can be used for targeted surveillance and both local or national prospective studies.

4.4. Most epidemiological studies on *Campylobacter* infection have analysed risk factors for *Campylobacter per se*. This genus is adapted to a wide range of ecological niches throughout the food chain. Microbiological data show that the prevalence of different *Campylobacter* species and subtypes varies between different potential sources of infection, including different animal species,

foods and water. In addition survival between the animal or environmental source and the food production process is likely to vary between subtypes. The organism is also extremely variable and any analysis of risk factors based on comparisons between different subtypes requires a large database for statistical validity. This study will generate such a database with comprehensive patient data, collected as early as possible after exposure, linked to detailed strain characterisation which can be extended with molecular fingerprinting for more detailed analyses.

4.5. Data on reported *Campylobacter* infections within a local area will be used in comparison with surveillance of food and environmental samples to analyse association between potential hazards and infection.

5. Future plans

5.1. The volume of data collected means that it will be possible to assess the relationships between different *Campylobacter* subtypes and a wide range of sources and vehicles of infection.

5.2. To test this approach a preliminary case-case comparison has been made designating patients infected with *C. Coli* were as a 'case' and those with *C. jejuni* as 'controls'. While such a comparison will not address risks common to both species, it will indicate differncial risk between the species. Analysis indicated that *C. jejuni* cases were more likely to be admitted to hospital than *C. Coli* cases (P=0.04) but there were no significant differences between *C. Coli* and *C. jejuni* cases in terms of age (P=0.08), gender (P= 0.59) or geographical distribution (P=0.15).
The univariate analysis indicated that cases of *C. Coli* infection were more likely to have travelled abroad, consumed offal, drunk bottled, river, stream or spring water in the fortnight prior to the onset of illness but were less likely to have eaten sausages than cases of *C. jejuni* infection. A multivariate analysis, after adjusting for confounding and simplification using the LR test, showed that cases of *C.coli* infection were more likely to have drunk river, stream or spring water, or unboiled tap water than cases of *C. jejuni* infection. They were less likely to have eaten pre-packed sandwiches. Further analysis of this model is underway and a paper in preparation. The model can also be extended to include analyses of risk factors between subtypes within either species.

5.3. Data from this study will be used to validate the typing approaches used by the CRU. New methods can be introduced as they become available and tested on subsets of well-characterised strains of known provenance. The clinical data will be used to assess possible associations of subtype with disease patterns and DNA based methods used to explore correlations between specific genes or alleles and severity of the presenting illness. There is also an opportunity to use the study data as the basis for studies on long term sequalae of *Campylobacter* infection including Guillain-Barré syndrome.

6. Conclusion

The sentinel surveillance system now in place will, for the first time, bring together detailed strain characterisation and full epidemiological data on a large series of patients. The scale of the project means that detailed analysis of *C. Coli* and *C. jejuni* subtypes can be used to generate hypotheses which can be tested by both epidemiological and microbiological methods. This will inform the development of strategies designed to reduce the incidence of *Campylobacter* infection and provide a means of testing their efficacy.

7. Reference

Wheeler, J.G., Sethi, D., Cowden, J.M., Wall, P.G., Rodrigues, L.C., Tompkins, D.S., Hudson, J. and Roderick, P.J. (1999) Study of infectious intestinal disease in England, rates in the community, presenting to General Practice and reported to national surveillance. *British Medical Journal* 318, 1046-1050.

Table 1: Campylobacter *isolates referred May-July 2000*

Predominant subtypes – *C.coli* - total 347

Serotype	Phage type	Number	%
56	44	47	13.5
56	2	34	9.8
66	2	29	8.4
26	44	16	4.6

Predominant subtypes - *C. jejuni* - total 4,614

Serotype	Phage type	Number	%
Untypable	1	711	15.4
50	5	161	3.5
50	34	143	3.1
Untypable	33	134	2.9
50	1	106	2.3
Untypable	2	80	1.7
23	1	75	1.6
37	1	72	1.6
12	2	72	1.6
12	Untypable	65	1.4
Untypable	Untypable	63	1.4
2	33	56	1.2
12	1	54	1.2
8	1	51	1.1
21	8	47	1.0

Table 2: Summary of risk factor analysis for first three months data

Exposure	Responses Total 1577					
	Yes		No		Not recorded	
Travel away from home						
Outside UK	297	19%	1249	79%	31	2%
Within UK	251	16%	1251	79%	75	5%
Foods						
Eaten rare	131	8%	1125	71%	304	19%
Unpasteurised milk	113	7%	1331	84%	133	8%
Bird pecked milk	23	2%	883	89%	84	8%
Water						
Private supply	84	7%	1200	76%	293	19%
River, stream, spring	34	2%	1286	82%	257	16%
Engineering/supply problem	110	7%	1397	89%	70	4%
Animal contact						
Any contact	917	58%	634	40%	26	2%
Farm visit	119	8%	924	59%	534	34%
Other patients						
Within the household	247	16%	1295	82%	35	2%
Outside the household	161	10%	1372	87%	44	3%

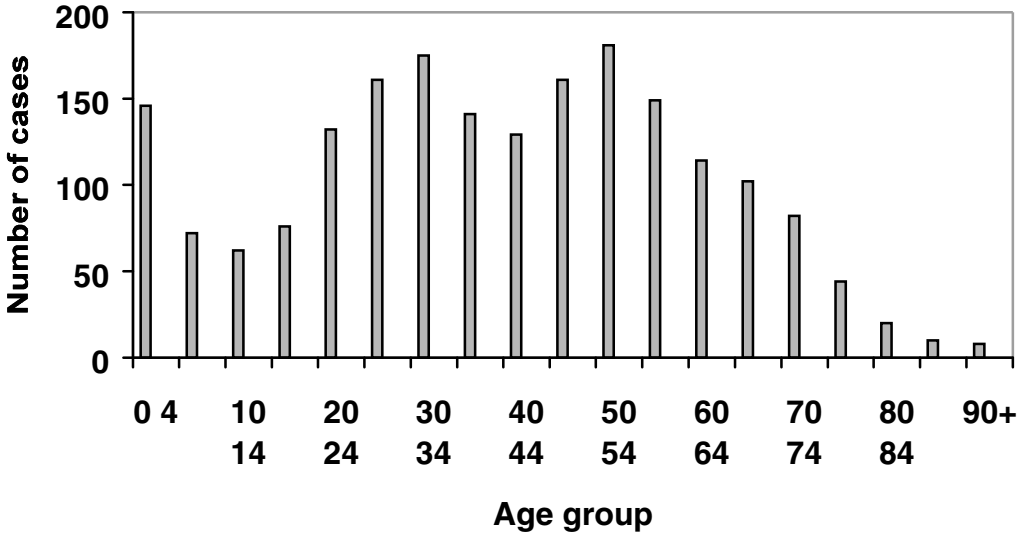


Figure 1: Age distribution of patients

13. Methods for isolation of *Campylobacters* from humans, animals, food and water

Frederick (Eric) Bolton

1. Introduction

There are numerous *Campylobacter* species but not all of these are important human pathogens. In developed Westernised communities *Campylobacter* infection is the commonest form of bacterial gastroenteritis. The major pathogens are known as the thermotolerant or thermophilic *Campylobacter* species. These include *Campylobacter* jejuni, *C. Coli*, C. lari, C. upsaliensis. In developing countries it is also possible to isolate other *Campylobacters* such as *Campylobacter* jejuni ssp. doylei. *Campylobacter* infection is a zoonosis and infection is usually acquired following consumption of contaminated food and water or following direct contact with farm animals. These organisms are part of the normal microbiological flora of the intestinal tract in all warm-blooded mammals and birds. Methods have therefore been developed for the isolation of *Campylobacters* from animal, food and clinical specimens.

Since the early 1970's several isolation methodologies have been developed. These range from the original centrifugation filtration methods developed by workers in Belgium to subsequent development of selective agar media and enrichment broth formulations. A summary of key developments is listed in Table 1.

2. Factors affecting growth and isolation

2.1 Selective culture media

Different basal media have been used to develop various formulations but it is necessary to add additional supplements to these basal media for optimal growth of *Campylobacter* species. Complex substrates such as blood, serum, charcoal and oxyrase have all been used successfully in culture media. Other workers have experimented with defined substrates and those currently in vogue include ferrous sulphate, sodium metabisulphate and sodium pyruvate (known as the FBP supplement), haematin, sodium thioglycollate, µ-ketoglutamic acid and occasionally catalase.

For the effective isolation of *Campylobacters* from animal, food and clinical samples it was necessary to develop selective culture media. Many antimicrobial agents have been used in media formulations. Antimicrobial agents that inhibit gram negative organisms include: cephalosporins, trimethoprim, polymyxins and novobiocin. Antimicrobial agents that inhibit gram positive organisms include vancomycin, teicoplanin, bacitracin, rifampicin and sodium deoxycholate. When culturing non-clinical samples it is also important to inhibit fungi and yeasts and traditionally cyclohexamide or amphotericin have been added to media formulations. Recent problems with the availability of cyclohexamide have meant that amphotericin has become more widely used. Antimicrobial agents have been used in various combinations by different workers to develop a range of selective agar media and selective enrichment broth formulations. Selective blood agar media in use, worldwide, include: Blaser-Wang agar, Butzlers modified agar, Campy- Cefex agar, Preston agar and the original formulation of Skirrow's agar. Subsequently blood free containing media have been developed. These usually contain charcoal and those in common use are charcoal cefoperazone deoxycholate (CCD) agar with amphoteracin, charcoal amphoteracin teicoplanin (CAT) agar and Karmali agar.

An alternative to selective agar media is the technique of passive filtration developed by Steel and McDermot in 1994. This method works on the principle that *Campylobacters* are selected by their ability to pass through membranes of pore size

0.65 or 0.45 microns. Drops of faecal suspension are placed on top of a filter membrane which is located on the surface of a blood-agar medium. This is left at room temperature for 45 minutes and *Campylobacters*, if present, pass through the membrane and onto the surface of the blood agar medium. The membrane is removed and the culture plates incubated microaerobically at 37°C for 48 hours or longer.

Many *Campylobacter* enrichment broth formulations have been developed but only a few of these have been adopted as standard methods in different countries. Most of these enrichment broths include blood as an additional supplement and usually the FBP supplement described above. *Campylobacter* enrichment broths adopted in various national standards include Doyle and Roman broth, Preston broth, Exeter broth, Park and Saunders broth and blood-free broth based on the charcoal formulations described previously.

2.2 Temperature of incubation

Thermophilic/thermotolerant *Campylobacters* can readily be isolated by incubation of culture media at temperatures between 35°C and 43°C. It is common practice, however, to use 42°C for selective agar plates and enrichment broths when isolating the thermotolerant *Campylobacters* or to incubate culture media at 37°C when isolating a wider range of *Campylobacter* species. Within Europe 41.5°C has been recommended in order to harmonise the temperature of incubation for isolation of *Salmonella*, E. *coli* O157 and *Campylobacters*. Because of the problem of sublethally damaged organisms some workers prefer incubation at dual temperatures, for instance, incubation initially at 37°C for several hours followed by incubation at 42°C.

2.3 Microaerobic requirement of *Campylobacters*

Campylobacter species are microaerophilic organisms and the most effective microaerobic conditions are those that contain 5-10% oxygen, 5-10% carbon dioxide and 5-9% hydrogen. These gaseous environmental conditions can be produced by a variety of different techniques. The evacuation-replacement method in conjunction with tank gases is still used but gas generating kits from several commercial sources are now common place. Variable atmosphere incubators which allow the user to produce a microaerobic atmosphere suitable for the growth of *Campylobacter* species are also available.

2.4 Factors affecting isolation of *Campylobacters*

The type, nature and composition of the sample to be tested can affect the ability to recover and isolate *Campylobacter* species. The numbers and types of competing micro-organisms in the sample can also have an effect. In non-clinical specimens the numbers of *Campylobacters* present can be low and the organisms may be sublethally injured. This can significantly effect the ability to isolate these organisms. The choice of culture media and the isolation protocol therefore needs to be optimised to meet the challenges of the various types of samples in which *Campylobacters* may be encountered.

3. Isolation from clinical specimens

Campylobacters are infrequently isolated from blood cultures during the bacteraemic phase of infection. Most commercial laboratory based culture media used for blood culture successfully isolate *Campylobacter* species. If *Campylobacter* bacteraemia or septicaemia is suspected it is important to subculture the broths to blood agar and to incubate these microaerobically at 37°C.

In the acute phase of infections *Campylobacters* can readily be isolated from faecal specimens. Some workers prefer to make a faecal suspension prior to inoculation of culture media but others prefer to inoculate faeces directly onto the culture medium. For the routine isolation of *Campylobacter* jejuni and *C. Coli* it is only necessary to inoculate samples onto a selective agar culture medium such as Skirrow agar or one of the blood free charcoal containing media. These are incubated microaerobically at either 42°C or 37°C for 48 hours. In a study using 1286 faecal specimens, cultured onto modified CCD agar and incubated microaerobically at 37°C for 48 hours, 177 specimens were found to be positive. Ninety-eight percent of these were positive following incubation at 37°C whereas 86% were positive following incubation of 42°C. The choice of selective agar culture medium and the method of producing the microaerobic atmosphere may significantly affect the recovery at different temperatures. Some workers have concluded that the duration of the incubation period can also have an effect on isolation from clinical samples. The results shown in Table 2 indicate that approximately 97% of positive faecal samples are detected after 48 hours incubation. Hence, there is little justification for extending the period of incubation for the isolation of *Campylobacter* jejuni and *C. Coli* from clinical specimens in a routine microbiology laboratory.

In developing countries where a wider range of *Campylobacter* species may be encountered in faecal specimens direct plating to a selective agar may not be optimal. In this case it is important to adopt the passive filtration method described previously. Although this method is less sensitive

for the isolation of *Campylobacter* jejun*i* and *C. Coli* it is the only effective method for isolating *Campylobacter* upsaliensis and *Campylobacter* jejuni ssp. doylei from faecal specimens. Culture plates are usually incubated at 37°C but extended periods of incubation can improve the recovery significantly (Table 2). Incubation for 4 or 5 days may be necessary to recover all *Campylobacter* species present. An alternative to the passive filtration method is to use charcoal amphoteracin teicoplanin (CAT) agar. This medium incubated microaerobically at 37°C was reported to successfully isolate *Campylobacter* jejuni and *C. Coli* and *Campylobacter* upsaliensis. Recent evaluations indicate that this medium does not recover all strains of *Campylobacter* upsaliensis because some strains are inhibited by the antimicrobial agent present.

Enrichment culture of faecal specimens is not usually necessary for routine microbiological diagnosis. However, if the sample is delayed in transit or has been taken in the convalescent phase of the illness or when examining samples from family contacts, enrichment culture can be a valuable adjunct. Culturing these types of samples can increase the yield by up to 15% of positive samples. This may be valuable in certain epidemiological based studies.

4. Isolation from animal specimens

Campylobacter jejuni and *C. Coli* are part of the normal gastrointestinal flora of many warm-blooded animals and birds particularly those used in the human food chain. The types of samples encountered may be faecal samples, intestinal contents, rectal swabs or cloacal swabs. In addition carcass swabs may be taken following evisceration at the abattoir or slaughterhouse. *Campylobacter* excretion in cattle and sheep varies depending on the season. Isolation can be achieved using direct selective agar media and by enrichment culture methods. Rectal swabs, cloacal swabs and carcass swabs are best cultured by enrichment techniques. The numbers of *Campylobacter* jejuni and *C. Coli* in the gastrointestinal tract of poultry is consistently higher than in other animal species. Direct plating may be the preferred choice of isolation method. For some studies it is important to enumerate the organisms in the gastrointestinal tract of animals. This can be done by preparing serial dilutions of the faecal or gastrointestinal samples and then inoculating 0.1 or 0.5 ml directly onto the surface of a selective medium or by using a most probable number (MPN) technique. Quantitative methods are particularly relevant when intervention strategies are being developed and evaluated.

For the isolation of *Campylobacter* upsaliensis and *Campylobacter* helveticus from dog and cat faecal specimens it is important to use the passive filtration method.

5. Isolation from food samples

The food samples most frequently tested for *Campylobacter jejuni* and *C. Coli* are poultry, red meats, shellfish and milk. It may also be necessary to examine vegetables particularly salad vegetables and fruit which may have become contaminated following irrigation with sewage contaminated water.

All of the meat samples can be tested by taking a defined mass of the food product or in the case of milk a defined volume. An alternative to taking a defined mass of sample is to take a surface rinse sample and this is widely adopted for sampling poultry carcasses and poultry meat. The rinse fluid is then cultured using an enrichment protocol or can be used to enumerate *Campylobacter* jejuni or *C. Coli*. An alternative with poultry is also to culture the neck skin as an indicator of the contamination rates of the carcasses. Liquid samples such as milk may require concentration and this is effectively done by centrifugation. It is then possible to culture the deposit from the milk and the cream layer separately.

Campylobacters present in food samples may have been subjected to a number of environmental stresses. They may therefore be sublethally injured due to freezing, following inadequate heat treatment or exposure to oxygen in air. Several approaches can be taken to aid the recovery of these sublethally damaged organisms in food samples. Primary growth in a non selective medium containing aerotolerant supplements will effect repair of oxygen damage to cells. The selective agents in culture media can also have an adverse effect on the recovery of sublethally damaged cells, particularly the presence of polymixin and rifampicin. Several workers have advocated delayed addition of some or all of the antibiotics used as selective agents. Incubation at a lower temperature 32°C to 37°C may also facilitate recovery of these damaged organisms. It is not possible to predict the outgrowth of damaged *Campylobacters* and therefore it has been suggested that prolonged incubation could have a significant effect on isolation. Incubation up to 96 hours for testing food samples has been suggested. With these factors in mind a number of different isolation protocols have been developed.

The current ISO/BS *Campylobacter* method recommends two procedures, one for culturing samples which may have been frozen and therefore

contain sublethally damaged organisms and one for culturing non-frozen samples. These procedures are illustrated in the flow diagrams in Figures 1 and 2. The procedure in Figure 1 is a standard enrichment protocol relying on subculture to one of the selective plating media. Alternatively the passive filtration method can be used to isolate *Campylobacters* from enrichment cultures. The second procedure is more complicated and is a multi-step process combining incubation in a microaerobic atmosphere, at a lower temperature with the addition of antimicrobial agents in a step wise manner. A number of evaluations of these methods have been undertaken with slightly different outcomes. Figure 3 illustrates the ability of three culture procedures to isolate *Campylobacters* from chicken, water and from milk. The procedure in Figure 2 was less successful than the more traditional enrichment protocols.

Studies undertaken by the PHLS in the UK has resulted in improved formulations and isolation of *Campylobacters*. The formulation known as Bolton broth (details given in Table 3) has been adopted by the FDA and by the USDA as the primary enrichment broth for isolation from food and from poultry respectively. This medium has been evaluated in separate studies against both the Preston broth and the Exeter broth and found to give superior isolation rates. Subsequently this broth has been modified as a blood free medium. The addition of the FBP supplement instead of blood has proved beneficial. Additional work undertaken at Exeter, Public Health Laboratory (UK), has modified the formulation by adding polymixin and rifampicin at reduced concentrations. The results of studies with this medium will be presented.

6. Conclusions

For the isolation of *Campylobacter* jejuni and *C. Coli* from clinical specimens, incubation of a selective agar at 37°C or 42°C for 48 hours is necessary. However, for the isolation of non *Campylobacter* jejuni/coli species passive filtration is the method of choice. Enrichment culture is only necessary in special cases.

For the isolation of *Campylobacters* from animal specimens direct plating may be satisfactory but enrichment culture can also be valuable. Quantitative estimation of the number of *Campylobacter* jejun*i* or *C. Coli* present may also be important in certain studies.

For the isolation of *Campylobacters* from food samples it is necessary to use enrichment broth formulations containing the FBP supplement. Enrichment broths should contain reduced concentrations of polymixin and rifampicin. It is preferable to incubate broths at 37°C for at least 48 hours and possibly up to 96 hours when culturing rinse samples or water samples.

Table 1. Historical Development of Isolation Methods

Year	Method	Authors
1972/73	Centrifugation/filtration	Dekeyser et al, Butzler *et al.*
1977	Selective agar medium	Skirrow
1977/78	Enrichment broths (Faeces)	Tanner & Bullin, Blaser *et al.*
1981/1982	Microaerobic gas kits	Bolton & Coates
1982/83	Enrichment broths (Food)	Oosterom, Park
1983	Blood free broth (Faeces)	Martin *et al.*
1983	Blood free agar	Bolton & Coates
1984	Blood free broth (Food)	Bolton *et al..*
1984	Passive filtration	Steele & McDermott
1989	Semi-solid medium	Goossens *et al*
1989	Variable Atmosphere Incubator	Bolton *et al.*
1991	Stepwise Protocol	Park & Saunders

Table 2. Effect of the Incubation Period on the Isolation of Campylobacters

Methods	Day 2	Day 3	Day 4
Mod CCD agar	173	176	177
Passive filtration	120	129	130

Bolton *et al*. 1988

Table 3. Formulation of Campylobacter Enrichment Broth (Bolton)

Composition	g/L
Meat peptone	10.0
Lactalbumin hydrolysates	5.0
Yeast extract	5.0
Sodium chloride	5.0
Sodium pyruvate	0.5
a-ketoglutamic acid	1.0
Sodium metabisulphite	0.5
Sodium carbonate	0.6
Haemin	10.0mg/l
Lysed horse blood	50.0ml/L

Selective Agents	mg/l
Cefoperazone	20
Vancomycin	20
Trimethoprim	20
Cyclohexamide	50

Figure 1. ISO/BS Campylobacter Isolation Procedure - 1

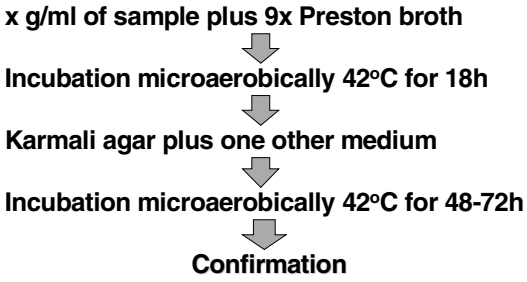


Figure 2. ISO/BS Campylobacter Isolation Procedure - 2

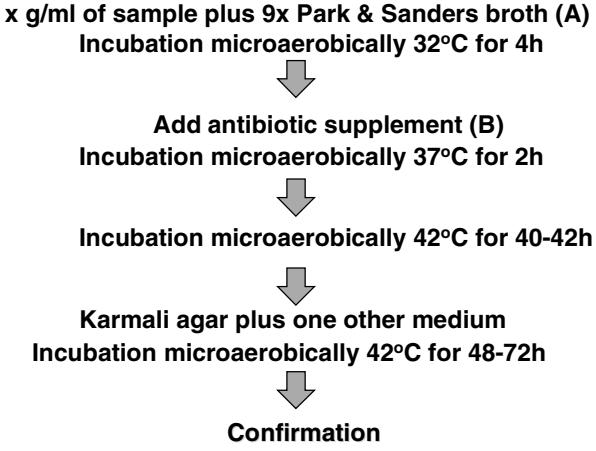
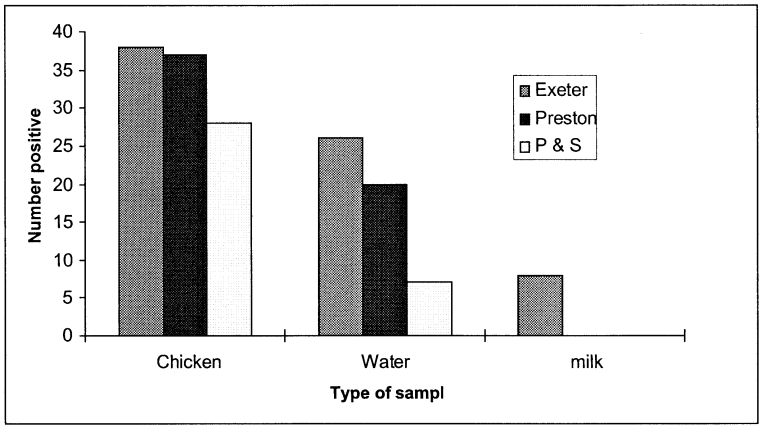


Figure 3. Isolation of Campylobacters Using Three Enrichment Media



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14. Methods for identification of *Campylobacter*

Peter A. R. Vandamme

Introduction

The taxonomic complexity of the genus *Campylobacter* has risen dramatically during the past two decades, thus inherently rendering identification more difficult. The recent history of these bacteria started in 1963, when Sebald and Véron (1973) transferred *V. fetus* and *V. bubulus* into a new genus, *Campylobacter,* because of their low DNA base composition, their microaerophilic growth requirements, and their nonfermentative metabolism. Ten years later, Véron and Chatelain (152) published a more comprehensive study on the taxonomy of the microaerophilic *Vibrio*-like organisms and considered four distinct species in the genus *Campylobacter*: *Campylobacter fetus* (the type species), *Campylobacter coli, Campylobacter jejuni,* and *Campylobacter sputorum* .

The 1970ies witnessed a first period of increased interest in these organisms due to the development of appropriate isolation procedures. The initial approach was based on a filtration method (Butzler et al., 1973), but the main breakthrough came a few years later after the development of a selective supplement that inhibited normal faecal flora (Skirrow et al., 1977). This simple isolation procedure thus enabled routine diagnostic microbiology laboratories to isolate *Campylobacters* and to evaluate their clinical role.

The availability of adequate isolation procedures led to a renewed interest in *Campylobacter* research during the early 1980ies. As a consequence, a manifold of *Campylobacter*-like organisms (CLOs) were isolated from a variety of human, animal, and environmental sources. Gradually , these CLO groups were identified as biochemical variants of established species or were described as novel *Campylobacter* species (Table 1). Also in the 1980ies, the concept of bacterial classification evolved considerably, not the least because of the technological progress in molecular biology, biochemistry, and several affiliated disciplines. One of the most prominent developments was the idea that bacterial classification should be based on natural evolution which is imprinted in the DNA sequence of highly conserved macromolecules. Using these new criteria, the taxonomy of *Campylobacter* and related bacteria was revised and the new genera *Arcobacter* and *Helicobacter* were proposed to accommodate several species previously known as *Campylobacters* (Goodwin et al., 1989; Vandamme et al. 1991).

The 1990ies witnessed a second period of increased interest in *Campylobacter* research because of the discovery of bacteria, now know as *Helicobacter pylori,* that were associated with a variety of gastric diseases. The potential link between various gastric and intestinal disorders and an infectious agent led to the discovery and description of a considerable number of novel species, some of which occur in animals as well as humans.

Nowadays, the genus *Campylobacter* comprises 15 distinct species, most of which have been isolates from human specimens. These human associated species include *C. fetus, C. hyointestinalis, C. sputorum, C. jejuni, C. Coli, C. lari, C. upsaliensis, C. helveticus* , *C. concisus, C. curvus*, *C. rectus*, *C. gracilis, C. showae* and *C. lanienae*. The genus *Arcobacter* comprises 4 distinct species. Of these, only *A cryaerophilus* and *A. butzleri* have been isolated from human clinical samples. Finally, the genus *Helicobacter* presently comprises 18 validly named species, but several additional novel species have been reported. *H. pylori, H. cinaedi, H. fennelliae, H. canis*, *H. pullorum* and bacteria referred to as "*Flexispira rappini*", are the species that are associated with human infections.

Identification of *Campylobacters*

A variety of different approaches have been used to identify *Campylobacters* to the species level. Detailed overviews were given by On (1996) and Vandamme and Goossens (1992). Together, the genera *Campylobacter, Arcobacter* and *Helicobacter* comprise over 40 species, the majority of which occur in human clinical samples. Below the general term '*Campylobacters*' is used to refer to species belonging to all three genera.

Whichever approach is used, it is important to cultivate these sometimes fastidious bacteria in optimal conditions in order to facilitate identification. Certainly, if non-genomic approaches are used, it is imperative that cells are grown in appropriate conditions. A microaerobic atmosphere comprising an increased level of carbon dioxide and a significant level of hydrogen (both in the range of 5 to 10%) will enable growth of all *Campylobacter*-like organisms. Some species like *C. concisus,* and some isolates of other species such as *C. hyointestinalis* and *H. cinaedi,* will not grow without hydrogen. An incubation temperature of 37°C is appropriate for all *Campylobacter* and *Helicobacter* species, but is suboptimal for *Arcobacter* species for which a temperature of 30°C is preferred. It should also be noted that although all *Campylobacters* grow in microaerobic conditions, some species like *C. gracilis* and *C. rectus,* should preferentially be grown in anaerobic conditions.

Classical phenotypic characteristics

The most widely adopted approach for the identification of *Campylobacters* is based on classical phenotypic characteristics, that include data derived from biochemical tests, cellular and colonial characteristics, and growth morphology. This approach is often contested as highly standardized procedures must be applied, and the discrimination amongst species often relies on one or two differential characters such as presence of hippuricase or urease activity (On and Holmes, 1995). Nevertheless, when this 'classical' approach is adopted by staff experienced in working with *Campylobacters*, the large majority of human clinical isolates can be accurately identified to the species level. This is exemplified by the approach often referred to as the Cape Town protocol (le Roux and Lastovica, 1998).

Cellular fatty acid analysis

Analysis of the cellular fatty acid components has successfully been used to identify many different bacteria to the species level (Vandamme et al., 1996). The total cellular fatty acid methyl ester composition is a stable parameter provided highly standardised culture conditions are used. Fatty acid methyl ester analysis is a simple, cheap and rapid method that reached a high degree of automatisation. Several authors used cellular fatty acid methyl ester analysis for the differentiation and identification of *Campylobacters*. One of the general conclusions was that some species, notably *C. jejuni* and *C. Coli,* could not be differentiated without additional biochemical data. Recent data presented during several *Campylobacter* conferences indicated that all other *Campylobacters* that occur in human clinical samples can be readily identified.

Protein electrophoresis

Comparison of whole-cell protein patterns obtained by highly standardised sodium dodecylsulphate polyacrylamide gel electrophoresis has proven to be extremely reliable for the identiciation of *Campylobacters* (Vandamme et al., 1996). To date, it is the only approach that successfully differentiated all culturable species within this bacterial lineage. However, in order to run the patterns in a sufficiently standardized way, the methodology is very laborious, time-consuming and technically demanding, and therefore, it is not appropriate for routine diagnostic laboratories.

DNA probe or PCR based identification assays

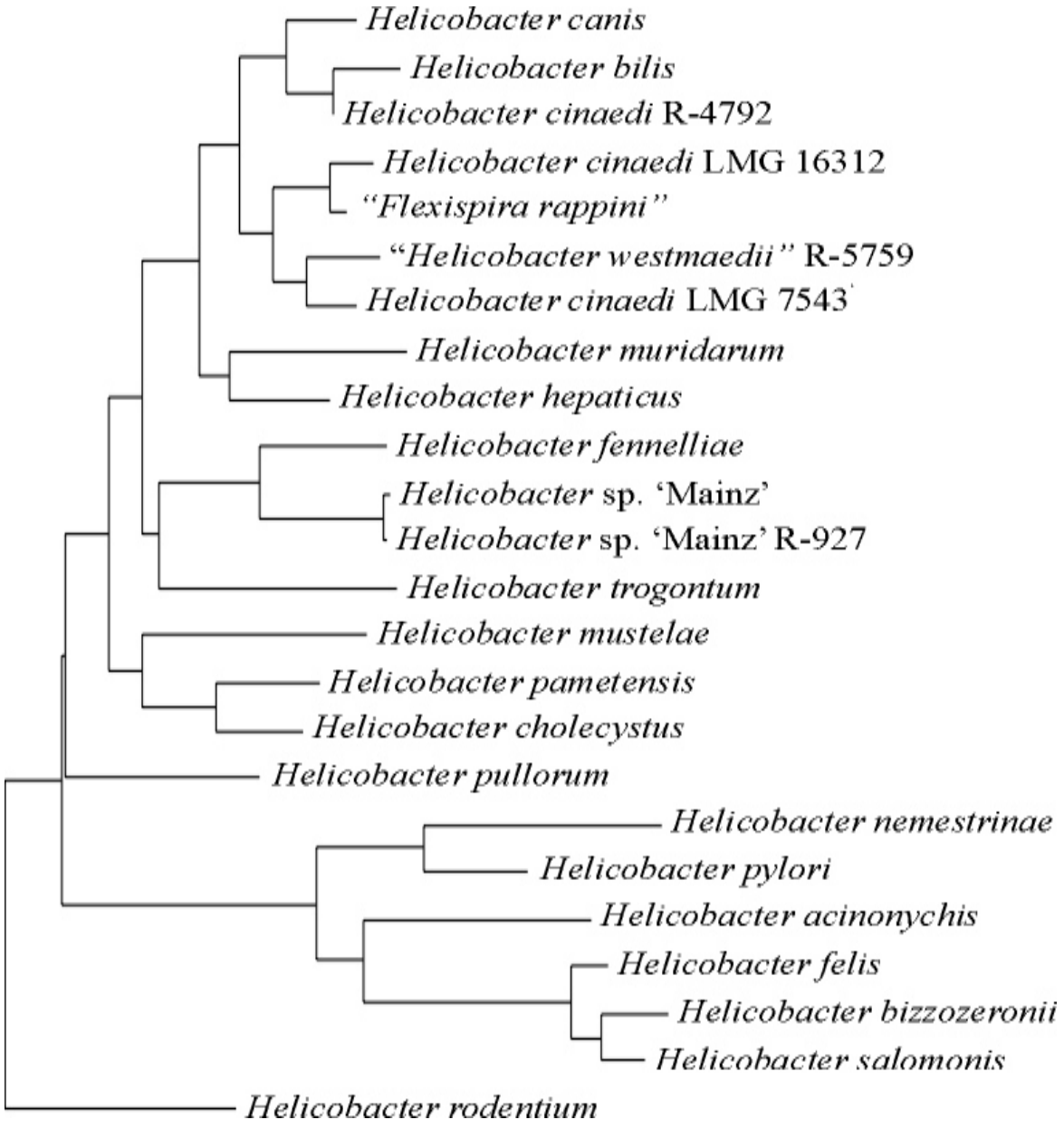
A variety of DNA probe and PCR based identification assays have been described for *Campylobacter* and *Arcobacter* species. These assays include tests for *C. fetus* (Ezaki et al., 1988; Wesley et al., 1991; Chevrier et al., 1989; Blom et al., 1995; Bastyns et al., 1994; Eaglesome et al., 1995; Hum et al., 1997); *C. hyointestinalis* (Gebhart et al., 1989; Wesley et al., 1991; Chevrier et al., 1989; Bastyns et al., 1994); *C. mucosalis* (Gebhart et al., 1989; Bastyns et al., 1994); *C. concisus* (Bastyns et al., 1995); *C. sputorum* (Bastyns et al., 1994); *C. jejuni* (van Doorn et al., 1997; Stucki et al. 1995, Occhialini et al., 1996, Day et al., 1997, Gonzalez et al. 1997, Linton et al. 1997, Vandamme et al. 1997), *C. Coli* (van Doorn et al., 1997; Gonzalez et al. 1997, Linton et al. 1997, Vandamme et al. 1997), *C. lari* (Oyarzabal et al., 1997; van Doorn et al., 1997; Linton et al. 1996), *C. upsaliensis* (Lawson et al., 1997; van Doorn et al., 1997), *C. helveticus* (Stanley et al., 1992; Lawson et al., 1997) and *A. butzleri* (Bastyns et al., 1995; Wesley et al., 1995, Harmon and Wesley, 1997; Houf et al., 2000), *A. cryaerophilus* (Bastyns et al., 1995; Houf et al., 2000), and *A. skirrowii* (Bastyns et al., 1995; Houf et al., 2000). In general, for these species it is feasible to examine many reference strains in order to adequately evaluate the sensitivity and specificity of these different assays.

For helicobacters too, a variety of specific oligonucleotide probes and PCR assays have been described. These include tests for *H. pametensis* ,

H. pylori, *H. hepaticus, H. pullorum, H. bilis, H. trogontum* and *H. canis* (Dewhirst et al. 1994; On, 1996; Fox et al. 1995; Mendes et al., 1996). It should be noted that for most *Helicobacter* species, there is a lack of sufficient reference strains available from international culture collections, so that it is mostly not possible to adequately evaluate the sensitivity and specificity of these *Helicobacter* assays. In addition, the constant developments in the taxonomy of *Helicobacter*, warrants a continued re-evaluation of the specificity of such test systems.

Several broad-spectrum molecular identification schemata based on restriction fragment analysis of PCR amplicons derived from small or large subunit rDNA have been described (Cardarelli-Leite et al., 1996; Hurtado and Owen, 1997a&b; Marshall et al., 1999). In these approaches, conserved target molecule are amplified by means of a PCR reaction. The amplicon is subsequently digested by restriction analysis, and the resulting fragments are separated in an electric field. The obtained banding patterns are relatively simple. Comparative pattern analysis allowed to distinguish most culturable species but may be complicated by the presence of internal transcribed spacers (first reported by Van Camp et al., 1993).

A relatively novel molecular diagnostic approach is AFLP or 'amplified fragment length polymorphism' analysis. AFLP is a very versatile but complicated procedure that has been used for



Legend of figure 1: Neighbour joining phylogenetic tree of H. cinaedi *strains (arrows) and related bacteria based on 16S rDNA sequence comparisons*

identification and typing in different bacterial groups. In *Campylobacter,* it was demonstrated that it could indeed be used for both applications (On and Harrington, 2000; Duim et al., 1999, 2000) but it remains questionable if a single approach can be developed that will allow identification of all clinically relevant *Campylobacters*. In addition, the complexity and cost of the procedure will exclude it from appication in the diagnostic laboratory.

Sequence analysis of 16S rDNA

Sequence analysis of the ribosomal RNA genes became the ultimate tool to study bacterial phylogeny during the 1980ies and international databases have been constructed to collect sequences and to make them available to the scientific community. Nowadays, it is not uncommon to identify unknown organisms that are of particular interest by means of (near) complete 16S rDNA sequence analysis and comparison with sequences present in international databases.

There are several aspects of concern. Although unsurpassed in its capacity to reveal the phylogenetic neighbourhood of an unknown bacterium, comparison of entire 16S rDNA sequences is generally not adequate for the identification of strains to the species level. It has been reported that strains belonging to different species may have identical 16S rDNA sequences, and that strains of one species may have 16S rDNA sequences that differ up to 3% (Stackbrandt and Goebel, 1994). For *Campylobacters*, differences of up to 4.5% have been reported (Harrington and On, 1999; Vandamme et al., 2000). There is definitely a lack of knowledge, not only of the strain-to-strain variation within a species, but also of the inter-operon variation within a single strain. This is illustrated in figure 1 that shows the phylogenetic position of multiple *H. cinaedi* isolates. Analysis of near complete 16S rDNA genes is obviously not appropriate for species level identification, but is mainly useful as a first-line approach that reveals the taxonomic neighbourhood of unidentified isolates. Identification strategies such as those described above should be considered to confirm tentative identification results obtained by comparison of complete 16S rRNA genes.

Conclusions

There are over 40 different species that belong to the diverse group of *Campylobacters*. The large majority of these occur in human clinical samples. A classical approach based on biochemical test results, and cellular, colonial and growth characteristics, may suffice to identify the majority of human isolates. Particularly researchers with considerable expertise with *Campylobacters* will successfully identify some of the less common species by means of this approach as well. There is a choice of other identification approaches available with varying degrees of technical complexity. The strategy chosen to identify *Campylobacters* clearly depends on the expertise and databases present in individual laboratories, and on the number of isolates to be identified.

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Table 1. Overview of Campylobacter-like organisms and their present taxonomic designation

Vernacular name	Present affiliation
CLO-1	*Helicobacter cinaedi*
CLO-2	*Helicobacter fennelliae*
CLO-3	Unnamed *Helicobacter* sp.
CNW	*Campylobacter upsaliensis, Campylobacter helveticus* *Campylobacter jejuni* subsp. *doylei*
GCLO-1	*Helicobacter pylori*
GCLO-2	*Campylobacter jejuni* subsp. *doylei*
Ferret GCLO	*Helicobacter mustelae*
EF Group 22	*Campylobacter concisus*
EF Group 24	*Helicobacter cinaedi*
EF Group 25	*Helicobacter cinaedi*
NARTC	*Campylobacter lari*
NASC	*Campylobacter lari*
NNC	*Campylobacter jejuni* subsp. *doylei*
UPTC	*Campylobacter lari*
Aerotolerant *Campylobacters*	*Arcobacter cryaerophilus, Arcobacter butzleri Arcobacter skirrowii*
Free-living *Campylobacter* sp.	*Sulfurospirillum*sp.

15. Strengths and weeknesses of bacterial typing tools for the study of Campylobacteriosis epidemiology

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Introduction

Campylobacteriosis is primarily considered to be a foodborne disease. The targeted control of foodborne bacterial pathogens is largely dependant on the identification of sources and routes of transmission. However, because *Campylobacter* spp. are ubiquitous in the environment and outbreaks are rare, source tracing has proved difficult. Meat derived from most livestock, including poultry, pigs, cattle and sheep can all be contaminated with intestinal contents colonised with *Campylobacters*. The relative contributions of these potential sources, and others such as domestic pets, wild birds, wild animals and contaminated water, to human infections are, as yet, undefined.

For other bacterial pathogens like *Escherichia coli* or *Salmonella enteritidis* the availablity of methods to describe, or type, individual strains has enabled

- the identification of sources and routes of transmission
- the tracking of strains throughout the food-chain
- the recognition of temporal or geographical clusters indicating outbreaks and
- the investigation of changing trends as a consequence of environmental pressures over time

Although *Campylobacter jejuni* and *C. Coli* are known to be both phenotypically and genotypically diverse, the development and application of suitable typing methods has been fraught with difficulties. The basic requirements for suitable typing techniques are discriminatory power, ease of use, rapidity, cost and reproducibility. The relative importance of these properties may vary depending upon use. For example when dealing with large numbers of strains for surveillance purposes rapidity and cost might be considered more important than discriminatory power. A further advantageous property would be the ability to enable comparison of data between laboratories. However for identification of outbreaks discriminatory power becomes critical. In this review the typing methods currently available and commonly used for these *Campylobacters* will be briefly described. A detailed review of techniques has been recently published (27). The relative strengths and weaknesses of these techniques are summarised in Table 1.

Phenotypic techniques

Early studies indicated a wide variation in a range phenotypic properties in *Campylobacters*, including total protein profiles by SDS-PAGE, antibiotic resistance, phage sensitivity, antigenic profiles and fatty acid composition. Only a few of these properties have been successfully exploited as phenotypic markers for epidemiological purposes.

Serotyping

Serotyping has been the most widely used phenotypic procedure for typing *C.jejuni/coli* strains. There are two globally accepted and well-characterised serotyping schemes. The Penner scheme is based on heat-stable (HS) antigens using a passive haemagglutination technique (21).The Lior scheme is based on heat-labile (HL) antigens (14) using a bacterial agglutination method. The weaknesses of both techniques is the high number of untypeable strains and limited availability of the serological reagents In addition, in a recent comparison serotyping was considered to be one of the least discriminatory subtyping techniques for strains from a variety of sources (17). Non-typeability is a consequence of both incompleteness of the typing sera set and variation in antigen expression. Serotype-specific antisera are costly to produce and difficult to quality control. Because of these difficulties *Campylobacter* serotyping has been largely confined to a few reference

laboratories. Recently a new scheme (6), also based on HS antigens, has been developed. By modifying the antibody production and antigen detection methodologies improved reproducibility and turn-around times have been reported. Although the weaknesses of reagent availability, high non-typeability and insufficient discrimination within certain serogroups still remain, the low skills requirement and rapidity of the test results have made this method suitable for national surveillance purposes in the United Kingdom.

Phagetyping

Several phage typing schemes have been described for *C.jejuni/coli* and some phages are common to several schemes (19). The levels of discrimination are relatively poor with over 50% of strains in the 10 major phage types. In addition the availability of the phage collection and expertise required to perform test, limits the suitability of the method for general use. The major value of phage typing currently appears to be as an adjunct to serotyping increasing the typability levels significantly. Nevertheless the cost/benefit of having to use two typing methods on all strains is debatable.

Genotyping techniques

More recently subtyping methods have been developed based on the genetic content of *Campylobacters*. The major advantage of such genotyping techniques is their potential for universal availability and transportable information. Some of these techniques, like ribotyping, pulsed field gel electrophoresis (PFGE) and flagellin (*fla*-) typing are already in use in a number of laboratories (18).

Flagellin Typing (fla typing)

The flagellin gene locus of *C. jejuni* contains two flagellin genes (*flaA* and *flaB*), arranged in tandem. Because of the presence of both highly conserved and variable regions (16) this locus is suitable for restriction fragment length polymorphism analysis of a polymerase chain reaction product (PCR/RFLP). There are many methods described for this typing scheme differing in a variety of technical aspects (27) all of which can affect the profiles obtained. Thus there is an urgent need to standardise and harmonise *fla*-typing procedures so that universally recognised types can be identified. The recent identification of concensus primer sets, based on large numbers of *fla*-gene sequences may contribute to this standardisation (27).

Fla-typing has the advantage of speed, simplicity and cost so it is potentially a good screening technique for epidemiological surveys. The levels of discriminatory power are reasonable so it should identify possible outbreak-related strains and be suitable for strain tracking through the environment. However, *fla*-typing is susceptible to genetic instability as a consequence of recombination and natural transformation (1, 8, 24). The rate of occurence of such genetic events is unknown but may be driven by environmental pressures. The effects of exchange of DNA between the two *fla* genes (recombination without transformation) on subtyping can be minimised by separately generating and digesting the PCR products of both *fla*A and *fla*B but then pooling the digested products before electrophoretic separation. In epidemiological studies, using *fla*-typing in a layered strategy with any other subtyping method will probably be sufficient to detect recombination events in the *fla* genes after transformation. *Fla*-typing, though excellent for short-term outbreak investigation, as in poultry houses is unlikely to be suitable for global or long-term time-related epidemiological studies.

Pulsed field gel electrophoresis

The digestion of the bacterial chromosome by restriction enzymes, that are rare DNA cutters, has proved a useful molecular epidemiological tool. The DNA fragments obtained by such digestions vary in length of between 20-200 kb. To protect the whole DNA and the subsequent fragments from shearing the bacterial suspension is immobilised in agarose before lysis and subsequent enzymatic steps are carried out by passive diffusion into these agarose blocks. Such large fragments need to separated using special conditions in an electrical field which changes orientation in a pulsed manner (5). The resulting macrorestriction profiles (MRP) can be analysed to provide a subtype characteristic of the strain (28). The most commonly used restriction enzyme for *Campylobacters* is *Sma*I but the use of an additional enzyme, like *Kpn*I, can significantly increase discriminatory power (10). PFGE is now a widely used subtyping procedure. With the development of standardised conditions for the PFGE methodology the comparison of MRPs between laboratories has now become feasible. PFGE is the main subtyping tool for Pulsenet, a USA-based epidemiological network for foodborne pathogens (www. cdc.gov/ncidod/dbmd/pulsenet/pulsenet.htm). However PFGE is technically demanding and requires at least some specialist equipment. Although the dependence of PFGE on rare restriction sites located throughout the genome should make it less susceptible to genetic instability, recent observations suggest that the genome of *C. jejuni* may be subject to mosaic rearrangements. Such events are detectable by PFGE (23, 25, 26) But the frequency of occurence of such events is unknown.

Ribotyping

The *Campylobacter* chromosome contains three copies of the ribosomal RNA gene loci. Regions of strong conservation within the rRNA genes, with highly variable flanking these provide suitable targets for subtyping (20, 22). Usually a subtyping profile is obtained by Southern blot hybridisation of digested genomic DNA with a probe specific for rRNA genes. This ribotyping technique has the advantage of high typability and the equipment and skills required to undertake the work are widely available. However ribotyping has the disadvantage of being a relatively time consuming and complex technique which has poor discriminatory power because there are only three ribosomal gene copies. Nevertheless, ribotyping has now been automated (riboprinting) for *C. jejuni* and *C. Coli* strains, increasing the rapidly and reducing the staff resources required, and potentially enhancing reproducibility. The costs of such riboprinting equipment and consumables are high. This plus the low throughput and limited discrimination will severely limit its acceptability as an epidemiological tool.

Randomly amplified polymorphic DNA (RAPD) analysis

Arbitrary primers (typically, randomly designed 10-mers) can be used for the amplification of random DNA products under low-stringency PCR conditions. Since the efficiency of amplification varies between loci, PCR products of varying intensity are produced. The length of the product also varies depending on the sites primed. Various RAPD methods have been developed for *Campylobacters* (4, 7, 12). The major disadvantages of RAPD are the complexity of the banding patterns, and the extremely poor reproducibility of the technique (4, 9). Variations in thermal cyclers, template purity, and procedures probably contribute to the poor reproducibility. The technique has been modified to use primers specific for enterobacterial repetitive intergenic consensus sequences (ERIC). Such primers can be used under higher stringency conditions to slightly improve reproducibility. Combinations of an ERIC primer and a randomly-chosen primer can also be used. However combined RAPD/ERIC amplification still suffers from low reproducibility and cannot be recommended for routine use.

Amplified fragment length polymorphism (AFLP)

AFLP is based on the complete digestion of chromosomal DNA with two restriction enzymes, one with a 4-bp recognition site and the other with a 6-bp recognition site. PCR primers, are designed to amplify only those fragments flanked by both restriction sites. By incorporating, in the primers design, one or more specific nucleotides adjacent to the restriction site the complexity of the banding profiles produced can be reduced. The PCR products are generally detected by means of fluorescent-labelled primers. By separation of the PCR products in denaturing polyacrylamide gels, using an automated DNA sequencer, resolution levels of one nucleotide differences are achievable. AFLP has recently been developed for subtyping *C. jejuni* (3, 11, 13). The technique randomly samples a portion of the whole genome. The small fragments of DNA detected are unlikely to be susceptible to genetic rearrangements but give a high level of discrimination. These properties make the technique eminently suitable for outbreak investigations (15). The major disadvantage is that the methodology is complex, requires a high level of skill and is expensive to set up. It is, therefore, unlikely that AFLP will become a routine tool for subtyping except in reference facilities.

Nucleotide sequencing

As direct nucleotide sequencing has become more automated, the sequence analysis of variable genes, usually following PCR amplification, is now a feasible option for subtyping. Nucleotide sequencing has the advantage of being highly reproducible, easy to interpret and portable between laboratories. The *fla* genes of *Campylobacters* have short variable regions (SVR) which are extremely suitable for sequence analysis (8, 16). This technique is currently being applied for epidemiological purposes to track *Campylobacter* strains through the environment. Like AFLP the equipment and set-up costs are substantial and unlikely to be available for routine laboratory use but with the declining costs of commercial sequencing facilities the technique could be accessible for confirmatory purposes.

Most recently the sequence analysis of multiple housekeeping genes (multilocus sequence typing, (MLST)) has been developed for *Campylobacters* (2). The methodology is PCR-based and is not technically demanding but is expensive and time consuming. Analysis of data can be undertaken remotely using data bases set up at the University of Oxford. (www.mlst.zoo.ox.ac.uk). This approach provides highly discriminatory data which is best suited for investigating the population structure of *Campylobacters* and the evolutionary relationships between strains. It should be noted, however, that MLST is not designed to recognize or identify pathogenic subtypes and that it is not a general subtyping tool.

Conclusions

There is an obvious and increasing requirement for the subtyping of pathogenic *Campylobacters* for epidemiological purposes. The choice of technique in routine clinical laboratories, which need to subtype perhaps many thousands of human isolates per year, will be dictated largely by speed and cost rather than other criteria. It is clear that a layered strategy is required for such laboratories, with quick and simple techniques like serotyping and/or phagetyping acting as screening tests followed by more discriminatory tests, such as PFGE, to confirm strain relatedness. For research-based laboratories the requirements will be tend to be for greater discrimination and high typability though this may depend on the application. Most such laboratories would use genotyping methods. Many such genotyping techniques are now available for *Campylobacters* and these urgently need to be standardised and harmonised as interlaboratory research collaboration becomes more important. One European Union-based initiative (CAMPYNET) is currently addressing these problems (www.svs.dk/campynet). Such genotyping techniques should also be applied in a layered strategy to overcome the possibility of genetic instability. At the Veterinary Laboratories Agency, investigating the short term veterinary outbreaks and strains tracking through defined environments, like abattoirs, *fla*-typing is used as the initial screen after which strain relatedness is confirmed using PFGE and/or AFLP. In conclusion it is not yet possible to recommend one most suitable technological approach for subtyping *Campylobacters*. The choice is dependant on the resources and requirements of each laboratory. However with the increasing pressure to reduce food-poisoning standardised typing methods for *Campylobacters*, acceptable internationally, will undoubtedly be needed.

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16. Strategies for controlling *Campylobacter* spp. in poultry

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ABSTRACT

In the United States, *Campylobacter* spp. is considered to be the most frequent bacterial agent of human gastroenteritis in the United States. Broiler chickens are frequently carriers of *Campylobacter jejuni/coli* resulting in contamination of carcasses and resulting in human exposure. Consumer exposure to *Campylobacter* seems inevitable if poultry meat is not handled hygienically and/or if the meat is not properly cooked before consumption. We have interest in reducing *Campylobacter* infection during poultry production to diminish contamination of processed carcasses. Our first step to control *Campylobacter* is to identify what are the most important sources for broilers. Worldwide, the onset of colonization among intensively reared broilers does not occur until week three or four of production, at which time the majority of the individuals within the flock quickly become infected. This phenomenon can be explained if the original source is not present until week three of broiler production or, if our samples or sampling methods are inadequate. We present data indicating the latter explanation is accurate for United States poultry production. In a separate study, *Campylobacter* isolates from commercial breeder flocks, as well as from progeny, were characterized and compared by ribotyping and by DNA sequencing of the short variable region (SVR) of the flagellin A gene. Ribotype patterns were identical for *Campylobacter* isolates derived from related parent and progeny. Additionally, DNA sequence analysis provided strong evidence that isolates of *Campylobacter* from related sources were of clonal origin. This report provides important evidence that *Campylobacter* can pass from one generation to the next in broilers.

A UNITED STATES COMMERCIAL BROILER INDUSTRY EPIDEMIOLOGY STUDY

The pathways involved in *Campylobacter* infection of poultry must be resolved to control it. We conducted an in-depth study among 32 broiler flocks on eight different farms, belonging to four major United States producers. The farms were rigorously sampled over one complete calendar year. Overall, 28 (87.6%) of the flocks became *Campylobacter*-positive and only four remained negative throughout the 6-8 week broiler rearing period. In the majority of flocks, sampled at bi-weekly intervals, *Campylobacter*-positive intestinal samples were not detected until 4-8 weeks of age. However, we found only six of the flocks to be *Campylobacter* positive among the environmental samples before shedding of *Campylobacters* was detected in the birds. By contrast, some *Campylobacter* negative flocks came from houses having pre-contamination in the production environment but not effectively transmitting infection to the birds. These findings are considered in relation to United States husbandry practices and present uncertainty about sources of *Campylobacter* infection for poultry flocks. Birds were often transported to the processing plant in coops that were already contaminated with *Campylobacter* and the organisms were sometimes found in samples of scald water and chill water. After chilling, the proportions of *Campylobacter*-positive carcasses from different producers ranged from 21.0% to 40.9%.

In total, approximately 1,700 isolates of *Campylobacter* were collected from approximately 11,000 samples taken. Thus far, we have conducted subtyping of the isolates from over half of the flocks. We chose to use *flaA* short variable DNA sequencing as our means for isolate

discrimination. At present, sequences from the majority of animal production and animal processing isolates were identical and therefore, we considered these to be of clonal origin. Our data suggests that contamination of the broiler carcasses likely originate from the feces of the production birds. Exogenous environmental samples such as wild bird feces demonstrated as much as 22% difference from both animal production and processing isolates. These data suggest that exogenous environmental samples were infrequently or were not a contributing factor to contamination of the final product. Our data suggests that multiple clones of *Campylobacter* may be present within a flock but, the number of clones we isolated rarely exceeded two. The contributions of *Campylobacter* from exogenous sources to production animals and most importantly to the final product needs to be further evaluated, but appears to be limited. Frequently, following detection of the organism in bird droppings, other environmental samples (such as wall and fan swabs) would become contaminated. Although we attempted to sample representative flocks of broiler chickens, we do not suggest that these findings represent the entire industry within the United States or, throughout the broiler producing world. However, our inability to find the same subtype of *Campylobacter* prior to its detection in broiler droppings does suggest an entirely alternative explanation (below) for transmission may be of importance.

BREEDER AND BROILER TRANSMISSION

Campylobacter jejuni, a Gram-negative, microaerophilic bacteria, is presently believed to be the leading bacterial etiological agent of acute gastroenteritis in the human population. Handling and consumption of poultry or poultry related products are considered to be a major source for *Campylobacter* induced disease in humans. *Campylobacter* exists in an apparently commensal relationship with poultry. The pathways involved in the infection of poultry continues to remain unclear. Several suspected horizontal sources or vectors of infection have been studied and include environment of the poultry house, hatchery pads, litter, feed, water, personnel, small animals on the farm, flies, and rodents. However, the subject of *Campylobacter* transmission from the breeder flock to the broiler flock has generated considerable debate. Egg transmission from the breeder flock traditionally has been dismissed as a source of entry because of the inability to culture *Campylobacter* from hatchery samples or from newly hatched chicks. Recently however, several published studies suggest circumstantially that egg transmission from one generation to the next is possible. Although there are published reports that suggest egg transmission may occur, deficiencies in cultural methodology have made this phenomenon difficult to prove. We used ribotype analyses in conjunction with *flaA* SVR DNA sequence analyses to provide evidence to suggest that *Campylobacter* can be transmitted from breeder flocks (parent) to broiler flocks (progeny).

Campylobacter isolates from three breeder hens and their respective progeny were analyzed. Isolates of *Campylobacter* obtained in Arkansas were collected from fresh fecal droppings from either the broiler breeder flock or from the respective progeny (a 6 week old commercial broiler flock reared approximately 20 miles from the parent). Ribotype analyses were performed for preliminary screening of the *Campylobacter* isolates. PstI ribotype analysis classified all of the Arkansas isolates as having the same ribotype pattern. In an effort to further substantiate this finding, short variable region (SVR) *flaA* DNA sequence analysis was conducted. The sequence data was analyzed and a dendogram was generated. The Arkansas *Campylobacter* isolates possessed identical *flaA* SVR DNA sequences. Both PstI ribotype analysis and SVR *flaA* DNA sequence analysis suggested that the epidemiologically related Arkansas isolates were also genetically related and were most likely of clonal origin.

Campylobacter isolates found in the Georgia study were obtained from fresh fecal droppings from broiler breeder hens, located within the same breeder facility, and from processed carcasses of their progeny. PstI ribotype patterns were identical among Georgia breeder and Georgia progeny isolates. Additionally, PstI ribotype patterns were identical for breeder and progeny. SVR *flaA* DNA sequence data from isolates of *Campylobacter* originating from the Georgia breeders and Georgia broilers, were identical.

One likely explanation for differences in ribotypes and SVR *flaA* DNA sequence is that a number of clones of *Campylobacter* are present within a broiler facility and that these particular isolates may represent a distinct clone. Distinct clones may originate from vertically transmitted sources, from horizontally transmitted sources, or from a combination of the two. A second possible explanation is that the Georgia broiler isolates were obtained from carcass rinses, and therefore, these particular isolates may have been contaminants originating from the processing environment.

Thus far, little has been published regarding the mode of infection and the spread of *Campylobacter* in poultry flocks. Additionally, much of what has been reported is inexact and is therefore misleading. In general, *Campylobacter*

colonization of broiler flocks is believed to originate primarily from a combination of animal sources: (a) farm animals other than broilers present on the broiler farm (b) farm animal sources outside the broiler farm and (c) domestic pets and vermin. For a variety of reasons (inadequate cultural methods for the recovery of *Campylobacter* from samples or inadequate sample size possibly due to a very low frequency of transmission) early studies strongly suggested that the vertical transmission route from hens to chicks was unlikely. Therefore, present intervention strategies focus primarily on control of *Campylobacter* at the farm, or at slaughter. Our findings provided strong evidence that the epidemiologically related isolates of *Campylobacter* from breeders and progeny were of clonal origin and that suggests *Campylobacter* can pass from one generation to the next in broilers. Therefore, intervention strategies will have to aggressively target locations previously excluded (breeder flocks, hatching cabinets, and hatchery environments). Epidemiological information is necessary to provide a basis for refining or adjusting intervention strategies to produce safer poultry food products.

ICELANDIC STUDIES

In another study, we are determining the relationship of *Campylobacter* originating in poultry in the transmission to humans among the Icelandic population. We are conducting this study in Iceland because of the unique opportunity provided by access to all human isolates from a controlled island which produces all of its own poultry. We have visited poultry production and processing facilities, and in general, substantial similarities exist in the U.S. poultry industry operations. Poultry consumption patterns in Iceland are roughly one-fourth the amount consumed in the United States but, the frequency of human Campylobacteriosis (per 100,000 persons) is several fold higher than seen in the U.S. This particular observation calls to question whether poultry is the most important source in human infection.

Thus far, we have been comparing *Campylobacter* isolates from prospective poultry carcass rinses to subsequent human isolates. All the human isolates of the organism have been gathered at Iceland's National University Hospital and the poultry rinse isolates were obtained from the processing plant and both sets were characterized by gene sequencing. In a limited subset of all the isolates obtained in August and early September, 1999, we noted identical gene sequences (of the human and poultry isolates) from the short variable region of the *Campylobacter* flagellin A genome. We sampled a large number of fully processed poultry carcasses in Iceland. Gathering such prospective data shall provide the risk assessment for human infection by transmission from poultry bome pathogens. The etiologic fraction for human infection via poultry will be assigned in this study. In subsequent studies we hope to determine what interventions for poultry can be practically achieved and how that intervention impacts human disease.

CONCLUSIONS

1. Within 32 flocks produced by the United States commercial broiler industry, environmental samples yielded *Campylobacter* that differed from those isolates we found on the fully processed carcasses. Environmental isolates that were found prior to broiler excretion of *Campylobacter* did not result in colonization. Environmental contamination occurred after the birds began excreting the organism. Occasionally, subtypes isolated from transport coops resulted in contamination of processed carcasses.

2. We present data supporting the role of the fertile hatching egg in the transmission of *Campylobacter* to the broiler flocks and the processed carcasses. The importance of this mode of transmission still needs to be assessed and may not be consistent between countries.

3. Iceland provides a unique opportunity to conduct quantitative risk assessment data collection opportunities. Models developed from this study should assist in creating appropriate intervention resolutions. Gathering such data is simpler in this small and well controlled production environment and, consequences of *Campylobacter* control in broilers can be monitored by decrease in exposure and frequency in human disease.

Table 1. Environmental samples found to be Campylobacter*-positive prior to the appearance of flock infection.*

*Flock code	Week(s) before infection detected	Positive samples (%) in week(s) preceding infection
AHF	4	Drag swabs (100)
ALS	4	**Mouse rinse (100), insects from house (25.0)
ALF	0	Wild bird feces (50.0)
DLW	4	Domestic animal feces (100)
DLS	2	Domestic animal feces (100)
DLSu	4	Drag swabs (100)

*Initial letter denotes producer code A-D; second letter indicates flock on high (H) or low (L) performance site; final letters correspond to winter (W), spring (S), summer (Su) and fall (F). NA, not applicable; ND, no data; NF, not found. ** Animals caught externally

Table 2. Environmental samples found to be Campylobacter*-positive for flocks that remained free from* Campylobacter*.*

*Flock code	Positive samples (%) during rearing period
BHW	Drag swabs (66.6), fan swabs (50.0)
CHW	**Mouse intestines (50.0), wild bird feces (50.0), standing water (100)
CLW	Wild bird feces (50.0)

*Initial letter denotes producer code A-D; second letter indicates flock on high (H) or low (L) performance site; final letters correspond to winter (W), spring (S), summer (Su) and fall (F). NA, not applicable; ND, no data; NF, not found. **Animals caught externally.

17. Strategies for post-harvest control of *Campylobacter*: A Review

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Introduction

Various bacteria belonging to the family Campylobacteraceae can be isolated from poultry products. Species include *Campylobacter jejuni* subsp. *jejuni* (referred to here as *C. jejuni*), *C. Coli*, C. *lari* and *C. upsaliensis, Arcobacter butzleri*, *A. cryaerophilus* and *A. skirrowii, and Helicobacter pullorum*. The majority of these organisms are potential human pathogens, but *C. jejuni* is the most important as a cause of human enteritis and has been studied most widely in relation to poultry. Evidence suggests that poultry meat is the main source of human *Campylobacter* infection in those countries studied. The presence of the organisms on poultry products derives from symptomless carriage in the live bird. Although the caecum is the main site of colonisation, *Campylobacters* can be found throughout the alimentary tract, including the crop in some freshly slaughtered birds.

Contamination of poultry products with *C. jejuni / coli* (the two are not usually distinguished from one another) is widespread. The proportion of contaminated items varies from one batch of product to another and can be anything between 0 and 100%. Such variation is also seen in published surveys. Data from 14 countries (Waldroup, 1996) show that *Campylobacter*-positive products ranged from 1.8 to 100%, with counts up to 10^7 per carcass in the case of uneviscerated birds. Contamination occurs over the entire surface of the eviscerated carcass and, with freshly slaughtered birds, *Campylobacters* were isolated from 89% of neck skins, 93% of samples from the abdominal cavity and from 75% of samples taken from under the skin (Berndtson *et al.*, 1992). Occasional muscle samples were also positive, indicating a systemic infection.

The higher recoveries of *Campylobacters* in more recent studies are thought to be a reflection of the distinct improvements that have been made over the last 20 years in methods of isolation. It should also be noted that recoveries are higher from fresh rather than frozen poultry since *C. jejuni* is sensitive to freezing and this reduces both the proportion of positive products and the levels of *Campylobacter* contamination. Freezing can reduce counts by ten-fold or more; however, freezing of products and then thawing them for sale as fresh items would not be allowed in the European Union (EU).

The best approach to controlling product contamination is debatable. One view is that control during rearing is essential to reduce the *Campylobacter* burden in the processing plant. However, in the absence of any fully effective intervention measures at farm level, another possibility is that more attention should be given to the processing operation, including the use of treatments developed specifically to reduce carcass contamination. Because *Campylobacters* are intestinal in origin, emphasis must be given to limiting faecal contamination of carcasses. The problem is exacerbated by the fact that levels of intestinal carriage in the birds are generally much higher than those for other foodborne pathogens, with the consequence that carcass contamination is correspondingly greater.

This paper will consider factors involved in the spread and survival of *Campylobacters* during processing and work done on possible intervention measures in the plant. The feasibility of improving in-plant control will also be discussed.

Behaviour of *Campylobacter* in processing

During processing, microbial contamination of the carcass surface is significantly reduced, provided that hygienic practices are maintained.

Table 1 gives data from Mead *et al.* (1995) and shows that *Campylobacters* follow this pattern, although the majority of carcasses from carrier flocks may remain *Campylobacter*-positive throughout the process. In some studies, plucking and evisceration have clearly contributed to carcass contamination but, contrary to earlier views, the temperature of scalding appears to have little effect, even at temperatures around 60°C, where survival in the scald-water is relatively brief. When *Campylobacters* are present on the skin of the birds, some will become attached and these are not readily removed by any washing process. Such organisms are more heat-resistant in the attached state and able to survive the usual scalding regimes (Mead unpublished). They have also been shown to survive adjustment of scald-water pH to 9.0, which enhances heat-destruction in the water during 'soft' scalding of carcasses at 50°C (Humphrey and Lanning, 1987).

Despite the presence of attached cells, use of water immersion chilling may lead to lower counts on finished carcasses and experience suggests that reductions of about one $\log_{10}$ unit can be expected. Water chilling is less common now within the EU, but is the method of choice in the USA and various other countries.

Because carrier flocks introduce large numbers of *Campylobacters* into the processing plant, equipment, working surfaces, process water and the hands of operatives become readily contaminated. Aerial dispersion of the organisms in some parts of the plant adds to the problem and cross-contamination of carcasses is inevitable, even though there is usually a net reduction in numbers on the final product. Whether cross-contamination is important when carrier flocks with large numbers of *Campylobacters* are being processed, will depend upon the existence of pathogenic and non-pathogenic strains of *Campylobacter jejuni*. It is likely that not all strains are of equal public health significance and if those that are pathogenic to humans are in the minority, their spread during proceeding would increase the hazard. When processing of carrier flocks is followed by the arrival of a *Campylobacter*-negative flock, cross-contamination is always likely to occur (Table 2) and the contamination may persist from one processing period to another, if routine cleaning and disinfection of the plant are inadequate. In this context, mention should be made of the crates in which live birds are transported from the farm to the processing plant. The crates are rarely cleaned and disinfected properly at the plant and thus may transmit *Campylobacters* among flocks being transported (Jacobs-Reitsma and Bolder, 1998).

Table 1 **Campylobacter contamination in flocks sampled randomly over several days at a UK processing plant.**

Flock no.	Carcasses		Caeca
	after slaughter	after packaging	
1	*3.7 (100)	1.8 (90)	6.2 (100)
2	4.0 (100)	1.0 (80)	6.2 (100)
3	3.9 (100)	1.4 (87)	6.6 (100)
4	3.8 (100)	1.2 (93)	6.8 (100)
5	3.4 (100)	2.1 (100)	7.6 (100)

*Log_{10} cfu/g of neck skin or caecal content (geometric mean)

( ) Percentage of samples positive (n = 10 – 15)

Table 2 **Cross-contamination to campylobacter-negative birds after processing of a carrier flock (Genigeorgis *et al.*, 1986)**

Birds	Scald overflow	Plucker water	Caeca	Chiller overflow	Hearts	Livers
Carriers	*1 / 3	3 / 3	5 / 5	1 / 1	4 / 4	3 / 3
Non-carriers (farm A)	0 / 3	1 / 3	0 / 6	3 / 3	1 / 3	2 / 3
Non-carriers from farm A, processed next day	3 / 3	3 / 3	0 / 5	0 / 3	3 / 3	3 / 3

*samples positive for campylobacter out of number tested

Isolation of *C. jejuni* on laboratory media requires incubation in a reduced-oxygen atmosphere. However, a number of reports have shown that the organism can adapt to grow under aerobic conditions and may be induced experimentally to multiply on chicken skin at ambient temperature (Lee *et al.*, 1998). Despite this, there is no evidence for growth in the processing plant or that strains of *Campylobacter* can colonise processing equipment, as has been shown for *Staphylococcus aureus*. The main concern, therefore, is to prevent or minimise carcass contamination through dispersal of the organisms from intestinal contents.

Use of super-chlorinated water

Where permitted, super-chlorination of process water is a useful aid to hygiene control in the plant. It is usually applied either in the form of chlorine gas or as the liquid, sodium hypochlorite. The former is acidic in water, while the latter produces an alkaline solution. The efficacy of chlorine in destroying bacteria depends upon the concentration used and the contact-time. Chlorine is most effective at low pH and at temperatures well above the chill range. In high-rate processing, however, the contact-time is usually short and, even in water immersion chilling, where hypochlorite may be added continuously to the water, both pH and temperature are less than optimal. The purpose of chlorinating process water is often misunderstood. Although regarded by some as a means of decontaminating carcasses, chlorine is rapidly inactivated in contact with the skin and any other organic material, and it appears to have little **direct** effect on carcass contamination, especially on organisms attached to the surface. The value of chlorine as a hygiene aid lies in controlling microbial contamination of the processing environment, including equipment, working surfaces and the process water itself. In this role, it is highly effective at appropriate concentrations and there is no risk of tainting the product at the concentrations normally used [up to 50 parts per million (ppm) total residual].

Data show that *C. jejuni* tends to be more sensitive to chlorine than *Escherichia coli*, although differences between the two were not always great or entirely consistent (Table 3). Nevertheless, chlorination of process water is relevant to the control of *Campylobacters* in just the same way as it is for other organisms (see below).

Decontamination of carcasses

Much research has been devoted to the development of treatments that would eliminate or reduce contamination of carcasses with foodborne pathogens, especially *Salmonella*s. The ideal treatment would be cheap and convenient to apply and have no adverse effect on the product. Should an effective treatment become available, however, it should not be used as an alternative to good hygienic practices in the processing plant. Decontamination is not easy at line-speeds of 6000 carcasses per hour or more, where only a brief contact-time is possible and the carcass cavity is not readily accessible. In experimental studies, various chemical and physical treatments have been examined (reviewed by Hinton and Corry, 1999). While a suitable physical treatment that would carry no risk of chemical residues in the product is most desirable, a fully satisfactory system for high-rate processing is not yet available. Some of the chemical treatments that have been tested against *Campylobacter* contamination are shown in Table 4. Their effects in reducing carcass contamination are not large, although, in the case of lactic acid, it has been noted that viability of the organisms continues to decline on chill storage, without necessarily reaching total elimination. The skin of the carcass may offer a degree of protection against these treatments.

Table 3 — **Relative chlorine resistance of *Escherichia coli* and strains of *Campylobacter jejuni* (Blaser *et al.*, 1986)**

Test organism	4°C / pH 8		25°C / pH 6	
	30 sec	300 sec	30 sec	300 sec
E. coli	*21.0	73.3	43.6	64.8
C. jejuni 1	ND	>80.0	31.4	45.4
2	38.4	66.1	66.5	74.2
3	60.9	>82.5	>81.8	>81.8

*Percentage kill (0.1 ppm of free chlorine) : mean of 2 – 6 replicates
ND not determined

Table 4	Some chemical immersion treatments to control naturally occurring campylobacter contamination on chicken carcasses.	
Treatment and conditions	**Mean log_{10} reduction**	**Reference**
0.5% acetic acid (50°C / 90 sec)	0.80	Stern *et al.* (1985)
0.5% lactic acid (50°C / 90 sec)	0.77	Stern *et al.* (1985)
10% trisodium phosphate (16 - 20°C / 15 sec)	1.31	Federighi *et al.* (1999)
*10% trisodium phosphate (10°C / 15 sec)	0.16	Slavik *et al.* (1994)
*10% trisodium phosphate (50°C / 15 sec)	1.49	Slavik *et al.* (1994)
* Following storage at 4°C for 1 day		

Table 5	Effects of process changes on neck-skin contamination with campylobacter, using flocks with similar levels of caecal carriage.		
No of flocks (birds)	**Caecal content**	**Neck skin**	
		after bleed-out	**after packaging**
Before changes			
5 (65)	*6.8 (100)	3.7 (100)	1.8 (91)
After changes			
4 (60)	6.5 (100)	3.9 (100)	1.2 (85)
* Mean of log_{10} cfu per gram : percentage of positive samples in ( )			

Effects of drying

C. jejuni is known to be quite sensitive to drying, although there is variation between strains in this respect and survival is affected by temperature and relative humidity (RH) (Doyle and Roman, 1982). Studies on the chilling of pork carcasses by forced-air ventilation have shown that *Campylobacters* tend to die off as the carcass surface dries and similar, but less consistent results were obtained from air-chilled poultry, sampled in the vent region (Oosterom *et al.*, 1983). When carcasses were subjected to an overnight period of forced ventilation at 1°C and an RH of 82 – 84%, naturally occurring *Campylobacters* were largely eliminated from the breast surface, but were readily isolated from neck skin samples and cavity swabs, sites which did not dry out to the same extent (Mead and Hudson, 1987). Some air-chilling systems incorporate a drying stage at 30°C, which dries the skin in order to extend shelf-life. It is possible that this process could be adapted to enhance die off of *Campylobacters*, but uniform drying of all parts of the carcass could be difficult and excessive loss of yield would need to be avoided.

Modification of the process as a whole

Two studies have examined the effect on *Campylobacter* contamination of modifying several parts of the process. One study (Mead *et al.*, 1995) was carried out in the UK, the other in the USA (Waldroup *et al.*, 1996). In the UK study, it was observed that processing reduced numbers of *Campylobacter* on skin samples by 10 – 1000-fold. To improve hygiene control generally, three different measures were taken. Firstly, chlorinated water-sprays (up to 46 ppm of free chlorine) were installed to spray the knife-blade of the automatic killing machine, the head puller and the conveyor belt to the evisceration line. Secondly, chlorine was added to water used in the plucking machines and that used in the evisceration machinery was increased from 10 to 40 ppm. Chlorine concentrations were also increased in the three-unit water chilling system and maintained at 23 – 38 ppm of total residual chlorine. A third measure was to prevent unnecessary contact between carcasses being processed and two surfaces that rapidly became soiled. Comparing carcasses before and after the changes (Table 5), it was found that

Campylobacters on packaged products were significantly reduced, although the mean difference was relatively small in practical terms.

The study of Waldroup *et al.* (1996) involved a combination of six modifications to the process which was evaluated in five processing plants. The changes included use of counter-flow scalding, addition of a spray washer after scalding, use of 20 ppm of chlorine in the post-plucking washer, the water used on the evisceration-line transfer belt and in the final washer; maintenance of 1 – 5 ppm of free residual chlorine in the overflow to the water chilling system. Levels of *C. jejuni / coli* were reduced significantly in four of the five plants and the proportion of *Campylobacter*-positive carcasses in two of the plants. The reduction in counts was 0.4 – 0.8 $\log_{10}$ units, similar to that achieved in the UK. In neither of these studies was it possible to determine the most effective of the changes made.

Conclusions

1. Although contamination of carcasses with *Campylobacter* is usually reduced during normal, hygienic processing, control of the organism is hampered by the current high incidence of positive flocks and levels of intestinal carriage.

2. Because of the present situation, modification of the process, within the constraints of available technology, are unlikely to reduce contamination dramatically.

3. Better methods of carcass decontamination are required. These may need to focus on particular susceptibilities of *Campylobacter*, eg to drying.

4. The limitations of present control measures in the processing plant highlight the pressing need for effective on-farm intervention strategies.

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Annex 4. COUNTRY REPORTS

As provided at the WHO expert consultation. The content of the country reports is solely the responsibility of the authors

Contents

1. Nigeria 117
2. Japan 118
3. Chile 119
4. United Kingdom 121
5. Belgium 128
6. The Netherlands 130
7. France 133

Campylobacter enteritis in Lagos, Nigeria

Coker, A.O., Isokpehi, R.D., Thomas, B.N. and Amisu, K.O.

Campylobacter jejuni and *Campylobacter coli* remain as the two species of the genus, which have been isolated from cases of *Campylobacter* enteritis in Lagos, Nigeria. Most symptomatic infections occur in children under 2 years old and characterised by diarrhoea which manifests clinically by watery offensive stool. *Campylobacter jejuni* is more prevalent than *C. Coli.* The isolation rates of *C. jejuni* from children with diarrhoea were 5.2% in 1984, 11% in 1989 and 16.5% in 1994. In these studies, the infection has a 2:1 male to female ratio and *Campylobacter* species were not recovered from asymptomatic children. Strains were sensitive to erythromycin and nalidixic acid. *Campylobacter jejuni* biotype I and serogroups 2, 4, 29 and 36 are most common. Seasonal variation has not been studied in detail but isolation rates appear to be more during rainy than dry season. Correlation between animal and human strains has been docum! ented using phenotypic and molecular typing methods suggesting a zooanthroponotic route of transmission in Nigeria. Campylobacteriosis is not routinely diagnosed in Nigerian hospitals and there are no national incidence data though the infection may constitute a substantial part of the cases of diarrhoea burden associated with food poisoning. A national survey is therefore imperative to determine the epidemiological situation, risk factors, distribution of strains in immunocompromised individuals, role of seasonal variation in disease pattern and strain diversity, resistance to antimicrobial agents such as quinolones among strains from farm animals, identification of species other than *C. jejuni* and *C. Coli* as well as cases of sequelae such as Guillian Barré Syndrome. The information arising from such a survey will contribute to global understanding of the epidemiology of *Campylobacters*.

Trends of *Campylobacter* enteritis in Japan

Naoaki Misawa

The incidence of Campylobacteriosis in Japan is compiled independently through each of following: (1) Notification to the Food Sanitation Division. the Ministry of Health and Welfare complying with the Food Sanitation Law (in the Statistics of Food poisoning). (2) reports by prefectural and municipal public health institutes (PHIs) on *Campylobacter* detection in examination of food poisoning outbreaks performed at PHIs and health centers (in the Infectious Agents Surveillance Report). (3) Individual reports to the Research Group for Infectious Enteric Diseases, Japan of *Campylobacter* enteritis patients admitted to infectious disease hospitals (IDHs) in Tokyo and designated cities (15 hospitals in 12 cities).

The Statistics of Food Poisoning. *Campylobacter*, designated as a food poisoning agent, is implicated in food poisoning most often after *Salmonella*, Vibrio parahemolytics, and Staphylococcus aureus. *Campylobacter* food poisoning cases in the whole country suddenly increased to 9,497 in 1985. Thereafter, the number of reports decreased to 948 in 1993. However, it increased once again after 1994 and has remained at between 1,500 and 2,000 to present. The annual incidents numbered less than 50 before 1995, but increased to 65 in 1996, 257 in 1997 and 553 in 1998. This increase is largely due to the current stream of notifications from some prefectures of

bacterial food poisoning episodes implicating no more than a single case.

Isolation reports from PHIs. Among the reports of isolation during 1995 through 1998, 87% mentioned the species differentiated; *C. jejuni* accounted for 97% and *C. Coli* a very small percent. According to the reports of *Campylobacter* isolation by month, the largest number of reports came out during April through July. This trend is similar to the number of incidents of *Campylobacter* enteritis in the United Kingdom and the United States. Outbreaks of *Campylobacter* food poisoning reported by PHIs during 1993 through 1998 totaled 169. Twenty outbreaks (12%) involved more than 100 patients, 18 (11%) 50 to 99 patients, 80 (47%) 10 to 49 patients, and 51 (30%), two to nine patients. In 49 (29%) of the 169 outbreaks, the source of infection was identified; chicken meat or chicken-meat containing dishes were most often incriminated in 39 outbreaks, drinking water in three, and school lunch in seven. According to the surveillance done by PHIs, *C. jejuni/* coli has frequently been isolated from chicken meat and surface swabs at poultry slaughterhouses, reflecting that *Campylobacter* food poisoning is often caused by contaminated chicken meat and through secondary contamination from it. *C. jejuni* isolates serotyped by the *Campylobacter* Reference Centers of PHIs during June 1996 through May 1998 numbered 590 from 51 food poisoning outbreaks and 1,163 from sporadic diarrhea cases. Among the former, 209 isolated from 19 episodes (37%) were type LIO7, followed by 43 of type LIO2 from eight episodes (16%). Among the later, 145 isolates (20%) were type LIO4, followed by 63 (8.6%) of type LIO7, 62 (8.5%) of type LIO1 and 52 (7.1%) of type LIO2.

Inpatient reports from IDHs. The age distribution of the 214 inpatients diagnosed as *Campylobacter* enteritis during 1995 through 1998 shows that the age group of 0-9 years accounted for 35%, 20-29 years accounted for 33%, 10-19 years for 17% and over 30 years for a low percent. Of the age group of 20-29 years, 63% of the cases were infected overseas. There were slightly mare male cases than female ones. The inpatients were to few to correlate the incidents with the season. Inpatients showed such symptoms as watery stool (90%), bloody stool (48%) and mucoid stool (25%). Abdominal pain was noted in 87% and vomiting in 38% of the patients. The maximum body temperature was 38.3C on average.

Campylobacter jejuni and *C. Coli* in Southern Chile: ecological distribution, virulence factors and antimicrobial susceptibility

Heriberto Fernández

Introduction: In the last decades, the thermotolerant species of *Campylobacter* (*C. jejuni* and *C. Coli*) have adquiered great importance in public health, specially as agents of human diarrheal disease. The epidemiological aspects involved in spreading of this bacterial group are complex and not well understood or studied in developing countries, where these bacteria sems to represent a public health problem not yet well defined. in Chile, there is not an accurate perception by the sanitary authorities of the real magnitude of the public health problem represented by these bacteria. Probably the same could occurr in others developing countries, particularly in Latin America.

Since 1984, in our laboratory, located in Valdivia city (Southern Chile), we developed several research lines in order to asses the ecological distribution, the presence of virulence factors and the antimicrobial suscpetibility of *C. jejuni* and *C. Coli* strains isolated from different sources in the 10th Region of Chile (41º 29' to 39º 47' Southern latitude). Some of the results obtained are presented in this communication.

Methods: 1632 fecal samples obtained from 190 diarrheic and 157 healthy children, 84 Healthy adults (63 workers from a slaughter-house and 21 lumberer), 214 dogs (150 stray and 64 pet dogs), 80 pigs, 300 cows, 95 sheep, 443 hens (150 free-renaging hens (SPF) obtained from families of low socio-economical level from the periurban zone of Valdivia city, 143 laying hens individually caged and feeded with commercial prepared food an potable water and 150 specific pathogen free hens managed under strictly controlled sanitary conditions at the Veterinary Medicine Faculty), 100 ducks, 104 pigeons and 100 sparrows; as well as103 river water samples and 110 chicken livers, were cultured for *C. jejuni* and *C. Coli*. Adhesion, invasiveness, enterotoxin and CLDT were determined in 439 randomly selected strains using Hep-2, CHO and VERO cells. Antimicrobial susceptibility was determined initially in 120 *C. jejuni* strains isolated from hens, using the double dilution agar method. Later, 53 *C. jejuni* strains isolated from hens, 108 isolated from humans and 44 isolated from dogs were studied by the E-test method.

Results: *C. jejuni* and *C. Coli* isolation rates were 11.6 and 4.7% in diarrheic children and 3.2 and 3.2% in healthy children. In adults, solely *C. jejuni* was isolated but only from slaughter-house workers 17.5%). In pigs, cows and sheep the isolation rates of *C. jejuni* were, 15.0, 25.7 and 12.6%, whereas for *C. Coli* they were, 55.0, 4.0 and 2.1%. The isoltion rate of *C. jejuni* in stray and pet dogs were 36.0 and 14.1%, respectively and for *C. Coli* they were 15.3 and 7.8%. In free-ranging, laying and SPF hens the isoltion frequencies of *C. jejuni* and *C. Coli* were 58.8 and 8.0, 22.4 and 7.0 and 6.2 and 1.3% respectively. In the samples obtained from ducks, pigeons and sparrows, *C. jejuni* was isolated in, 66.0, 6.7% and 32% whereas *C. Coli* was in, 7.0, 3.8 and 1.0%. *C. jejuni* positive samples were 8.7 and 26.5% for river water and chicken liver and 22.3 and 41.2% for *C. Coli*.

With regard to the virulence factors 88.0, 70.8, 41.5 and 74.2% of *C. jejuni* strains showed adherence and invasive capacities and were enterotoxin and CLDT producers. In *C. Coli* strains, these capacities were observed in 91.0, 73.1, 32.7 and 64.7%.

All the *C. jejuni* strains of human origin were susceptible to erythromycin, ciprofloxacin and gentamicin being 4.6 and 1.8% resistant to ampicillin and tetracycline respectively. In the first study (double dilution agar method) 13.3 of the strains isolated from hens were resistant to tetracycline and 6.7% to ampicillin, being all susceptible to erythromycin, ciprofloxacin and gentamicin. In the second study carried out with

the E-test method in *C.jejuni* strains isolated from hens, we found, for the first time, strains that were resistant to erythromycin (58,5%), ciprofloxacin (17.2%) and gentamicin (1.9%). The frequency of strains resistant to ampicillin and tetracycline was 15.1%. In the strains isolated from dogs, 9.0% were resistant to ampicillin but susceptible to the other four antimicrobial drugs tested.

Conclusions: In Southern Chile, *C. jejuni* and *C. Coli* are widely distributed in animal reservoirs being also isolated from river water and liver chicken. They are frequent agentes of diarrea in children and could be also isolated from healthy children. There exist a strong relationship between the sanitary environmental conditions and the carriage frequency in animals considered as reservoirs. In adults, the close contact with animals is a risk factor to acquire the carrier state. Resistance to ampicillin and tetracyclyne, at different levels, was found in the different groups of strains under study. In the last three years increased the frequency os resistant strains to these drugs in hens, where resistance to erythromicin, ciprofloxacin and gentamicin was observed for the first time in our region.

Strains from all the isolation sources express one or more of the virulence factors studied.

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Public Health Laboratory Service data for England and Wales: Human infections and *Campylobacter* from retail foods

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National surveillance systems for foodborne disease and the incidence of *Campylobacter* infection in humans

There are three main national surveillance systems for gastrointestinal infection in England and Wales operated by the Public Health Laboratory Service Communicable Disease Surveillance Centre (PHLS CDSC). These are:

- The statutory notifications of food poisoning
- The national surveillance scheme for laboratory confirmed infections
- The national surveillance scheme for general outbreaks of infectious intestinal disease (IID)

In addition there is close liaison with the PHLS reference laboratories (Laboratory of Enteric Pathogens, Food Safety Microbiology Laboratory and the Enteric Virus Laboratory), and with external bodies such as the Veterinary Laboratories Agency, the Food Standards Agency, the Ministry of Agriculture, Fisheries and Food and the Department of Health.

The main characteristics of each data source are outlined below.

1. Statutory notification of food poisoning

All doctors in clinical practice have a statutory duty to notify the proper officer of the local authority of all clinically diagnosed cases of diseases specified under the Public Health (Infectious Diseases) Regulations 1988. Food poisoning is one of the infections that is notifiable. In 1992 the Department of Health's Advisory Committee on the Microbiological Safety of Food (ACMSF) defined food poisoning as "any disease of an infectious or toxic nature caused by or thought to be caused by the consumption of food or water." This is a very sensitive definition of food poisoning which includes non-infective causes. Since notification of food poisoning does not require that a laboratory diagnosis be obtained, it is not possible to determine the number of cases of notified food poisoning attributable to specific organisms.

Notification data are collated nationally by CDSC on behalf of the Office for National Statistics (ONS). The collated data are published weekly in the Communicable Disease Report (CDR Weekly). Notifications of food poisoning in England and Wales have risen steadily since the early 1980s such that by 1998 more than 90,000 cases had been reported nationally (Figure 1).

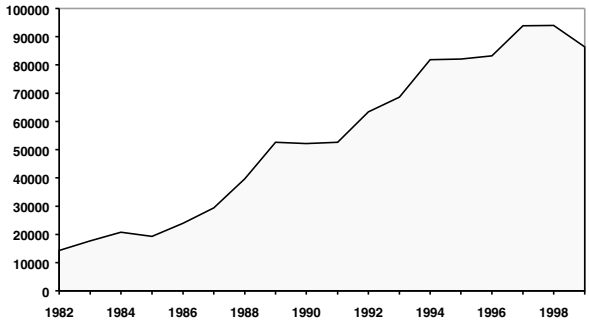


Figure 1: Food poisoning notifications: England and Wales 1982-1999

2. National surveillance scheme for laboratory confirmed infections

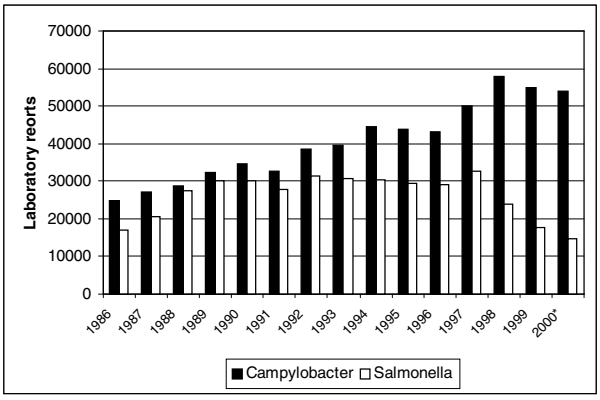
Clinical microbiology laboratories in England and Wales voluntarily report data on microbiologically confirmed cases of infectious disease to CDSC. The data reported include organism identification, specimen date and patient demographic data.

It should be noted that the following events must occur for cases to be included in the national surveillance database for laboratory confirmed infections:

1. an infected individual must consult a clinician (general practitioner or hospital doctor)
2. the doctor must arrange for a specimen to be taken and referred to a clinical microbiology laboratory
3. the laboratory must isolate or identify a pathogen
4. the laboratory must submit a report to the national surveillance centre

The national surveillance scheme for laboratory confirmed infections does not therefore include **all** infections caused by those pathogens under surveillance. The severity of the disease and the duration of symptoms associated with infection dictate both the proportion of cases that consult clinicians and the proportion of presenting cases from whom specimens are collected.

Despite these caveats, voluntary reporting of laboratory-confirmed cases is the most reliable means of determining trends in the major foodborne pathogens. The most noteworthy changes in reporting of laboratory confirmed infections in England and Wales were the continued rise in *Campylobacter* infection and a sharp fall in reporting of salmonellosis (Figure 2). *Campylobacter* continues to be the most commonly identified bacterial cause of gastro-enteritis in England and Wales and in 1997 the incidence exceeded 50,000 cases for the first time (www.phls.co.uk).



3. National surveillance schemes for general outbreaks of infectious intestinal disease (IID)

Since January 1992 enhanced surveillance of outbreaks of infectious intestinal disease has been conducted in England and Wales. General outbreaks are defined as outbreaks that affect people from more than one household. CDSC receives preliminary reports of general outbreaks of infectious intestinal disease (IID) from laboratories including the national reference laboratories, health authority Consultants in Communicable Disease Control (CsCDC) and local authority Environmental Health Officers (EHOs). Standardised questionnaires are sent to the appropriate health authority in order to collect a minimum dataset and the investigating CCDC is asked to complete the questionnaire when the outbreak investigation is complete. The completed questionnaires are returned to the national surveillance centre and the data entered onto a database.

Campylobacter is rarely recognised as causing foodborne outbreaks. Between 1993 and 1998 a total of 3712 general outbreaks of IID were reported to CDSC of which 1093 were foodborne (Table 1). The predominant causative organism in foodborne outbreaks of IID was *Salmonella* sp. accounting for 161 outbreaks in 1993 and 77 in 1998 [add 1999?]. In contrast *Campylobacter* outbreaks rarely exceed 10 *per annum.*

Table 1. Foodborne outbreaks – causative agents, England Wales 1993-1998
(Source: GSURV database CDSC)

	Year						
Organism	**1993**	**1994**	**1995**	**1996**	**1997**	**1998**	**1999**
Campylobacter	3	5	4	6	7	11	
Salmonella	161	107	104	101	153	77	
VTEC O157	6	0	5	7	4	4	
Grand total –all organisms	225	191	180	159	218	120	

The investigation of *Campylobacter* infection is often less rigorous than *Salmonella* infections which results in under-reporting of *Campylobacter* outbreaks. While over 90% of Local Authorities surveyed in a recent study, always investigated reports of *Salmonella* or *E.coli* O157 infection, only 63% always followed-up reports of *Campylobacter* infections (Rooney *et al*, 2000). Indeed *Campylobacter* scored lowest for follow-up of the 20 likely causes of sporadic food poisoning infections.

Despite the paucity of outbreak reports in the literature it is clear that there is a wide range of sources of infection. Chicken was implicated as the vehicle of infection in nine of the 46 *Campylobacter* outbreaks reported between 1996 and 1999 and water in four. Unpasteurised milk was identified as the vehicle of infection in two outbreaks.

Because *Campylobacter* are so widespread in nature, the source of *Campylobacter* contamination may not be the vehicle by which the outbreak is transmitted. Poor food handling practices may result in outbreaks where more than one pathogenic strain is transmitted. Nine of the above outbreaks fall into this category. For example, in an outbreak associated with a university fast food outlet (Gent *et al*, 1999) a total of 116 students and staff reported enteric illness over a five-week period. There was statistical evidence of an association between illness and eating at the implicated premises although a variety of *C. jejuni* subtypes were identified among strains submitted for typing. Environmental investigation identified a number of situations where thaw liquor from frozen chicken might contaminate surfaces used for the preparation of a range of foodstuffs. Thus, while the epidemiological evidence suggested a point source outbreak there were in fact a number of cross contamination events associated with multiple *C. jejuni* subtypes.

4. Reference laboratory referrals

Between April 1997 and March 2000 the PHLS Campylobacter Reference Unit provided a full reference service for two regions, with support for outbreak investigations throughout England and Wales. This generated typing data for approximately 16% of the infections reported nationally to CDSC. Typing is based on serotyping (Frost *et al*, 1998) and phagetyping (Frost *et al*, 1999) supplemented by Pulsed Field Gel Electrophoresis fingerprinting (Gibson *et al,* 1997) where further strain discrimination is required. Since May 2000 the regional system has now therefore been replaced with a sentinel system covering selected Health Authorities throughout England and Wales using standardised epidemiological and microbiological information from a representative sample of cases in England and Wales. The system, which is described more fully in a companion paper (Frost *et al*, this meeting) is designed to provide robust population denominators and provide a statistically valid sample from which extrapolation of trends is legitimate.

5. Campylobacter infection in the community

A recent Department of Health study into Infectious Intestinal Disease [IID] in England [Wheeler *et al*, 1999] estimated that there were 9.4 million cases of intestinal infectious disease in England each year, an incidence of 194 per thousand person years.

The data indicated that, of the estimated 8.7 campylobacter infections in every 1,000 person years, only 4.1 would consult a General Practitioner and only 1.7 are documented as laboratory confirmed campylobacter infections (Table 3). This would indicate that for every case currently reported there are a further 7.6 in the community or approximately 420, 000 cases per annum. Furthermore, while viruses were the most common cause of IID in the community, campylobacter was the most frequently identified target organism in the GP component of the study and were rarely isolated from symptomless controls (Tompkins *et al*, 1999).

6. Isolations from food

The PHLS carries out a number of structured surveys each year of a range of food items purchased from retail outlets. Many of the studies are in collaboration with the Local Authorities Co-ordinating Body on Food and Trading Standards (LACOTS). Since 1997 there have been seven such surveys, campylobacter being isolated in three surveys of raw foods. While the incidence in chicken products is not surprising the incidence in raw lamb or mutton in the halal meat survey is a cause for concern.

In a separate study looking at multiple campylobacter isolates from poultry portions and lamb, pork and ox liver almost 30% of samples yielded more than one species of *Campylobacter* or more than one subtype of *C.jejuni*. Recent data suggest that co-infection with multiple strains of *Campylobacter* species also occurs in 5-10% of cases acute human enteritis (Public Health Laboratory Service, unpublished data). The potential occurrence of multiple campylobacter species or subtypes in samples taken throughout the food chain further complicates the identification of links between animal and food contamination with campylobacter, and human infection.

Table 2: General outbreaks of Campylobacter *infection, England & Wales, 1996-1999*
Source: GSURV database CDSC

Year	Affected	At risk	Died	Vehicle[s]	Evidence
1996	8	9	0	Chicken (boiled/tandoori)	CC
	16	250	0	Lettuce, tomato	CC
	5	40	0	-	-
	9	15	0	-	-
	11	11	-	-	-
	33	140	-	Duck liver parfait	CO
	5	12	0	Raw milk	CO
	4	38	0	-	-
	12	-	0	-	-
1997	12	29	0	Chicken (stir fried)	CO D
	20	70	0	Sandwiches, vol-au-vent, chicken	CO D
	27	600	0	-	-
	34	220	0	-	-
	16	-	0	Water	D
	14	-	-	-	-
	22	62	0	Chicken (undercooked)	D
	22	98	0	Chicken	CO
	21	94	0	Chicken (cooked)	CO D
	11	13	0	-	-
	15	40	0	Not identified[¡]	CO
	52	3000	-	Mixed foods	CO
1998	4	-	-	Unpasteurised milk	-
	61	192	-	Curried meats, prawn salad	CO
	11	250	-	Water	D
	3	3	-	Chicken	D
	22	500	-	-	-
	30	10000	0	Lettuce, mayonnaise(garlic)	CO D
	8	126	0	-	-
	48	133	0	Prawn & salmon vol au vent	CO
	9	20	0	-	-
	20	20	0	Water	D
	12	-	-	-	-
	4	5	-	Mayonnaise	D
	11	18	-	Buffet	CO
	21	112	-	-	D
	24	53	-	vol au vents, sandwiches, pizza	CO
1999[¶]	10	384	-	-	-
	10	26	-	-	-
	9	30	-	Chicken liver pate	CO
	20	31	-	Bang bang chicken	CO
	12	32	0	Chargrilled chicken	CO
	25	61	-	-	-
	18	24	-	Spring water	M
	16	350	-	-	-
	24	100	-	-	-
	13	221	-	-	-

[*], More than one vehicle can be reported in each outbreak;
Evidence: D = Descriptive (strong circumstantial) evidence; CO = Cohort study;
CC = Case Control study; M = Microbiology
[¡], Outbreak associated with travel abroad (France);
[¶], provisional data.

Table 3: Infectious intestinal disease (IID) in England: reporting pyramids for disease in the community (after Wheeler et al, *1999)*

		Community	Presenting to General Practitioner	Positive by routine laboratory test	Reported to National Surveillance
All IID	Rate % reported	194	33.1 17.1%	2.1 1.1%	1.5 0.7%
Campylobacter	Rate % reported	8.7	4.1 47.1%	1.7 19.5%	1.1 7.9%
Salmonella	Rate % reported	2.2	1.6 72.7%	0.8 36.4%	0.7 31.8%
SRSV	Rate % reported	12.5	1.9 15.9%	0.012 0.9%	0.012 0.06%

Rates [per 1000 person years]: SRSV = small round structured virus

Conclusions

- Campylobacter is now by far the most commonly reported bacterial food poisoning pathogen in England and Wales.
- The vast majority of infections are reported as sporadic. Outbreak recognition is hampered by the lack of both microbiological and epidemiological follow-up of campylobacter infections.
- The widespread occurrence of campylobacter throughout the animal, food and water environments, and the possibility of cross contamination at the abattoir, retail outlet and the commercial or domestic kitchen, makes the tracing of sources of campylobacter infection difficult. The presence of more than one species or subtype of campylobacter in many samples compounds these problems.
- A lack of linked microbiological and epidemiological data means that the epidemiology of campylobacter infections in humans, and the role of the many potential sources of infection, is still poorly understood. Current surveillance initiatives in England and Wales are designed to generate data that will

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Table 4: National food studies; Campylobacter *isolated from foods on retail sale*

Study Programme	**Year**	**Food type**	**Positive for Campylobacter spp. (%)**	**Reference**
PHLS	Mar 1996 - Jul 1997	Raw cows' drinking milk	19 / 1205 (1.5%)	de Louvois J, Rampling A, 1998
LACOTS/PHLS	May-Jul 1997	Butcher shops: Raw meats (sausages, burgers) Cooked meats	 15 / 2330 (0.6%) 0 / 2330	Little CL, de Louvois J, 1998
PHLS	Jan - Feb 1998	Raw ewes' & goats' drinking milk	0 / 131	Little CL, de Louvois J, 1999
PHLS	Jan - Feb 1998	Imported whole unprepared lettuce	0 / 151	Little CL, et al. 1999
PHLS	Apr - May 1998	Halal butcher shops: Raw meats (mince, chopped/diced meats)	 52 / 183 (28%)*	Little CL, et al. 1999
LACOTS/PHLS	May - Jun 1999	Ready-to-eat burgers	0 / 3128	Little CL, Mitchell RT, 2000
LACOTS/PHLS	May - June 2000	Ready-to-eat organic vegetables	0 / 3552	Provisional data

* including 21/42 raw chicken products (50%) and 29/126 raw lamb/mutton samples (23%)

Campylobacter *isolated from meats on retail sale (after Kramer* et al*, in press)*

Source	Total examined	Positive for *Campylobacter* spp.	%	Samples with more than one species	Samples with more than one subtype
Chicken portions	198	165	83.3	4	35
Lambs liver	96	70	72.9	14	24
Ox liver	96	52	54.2	10	8
Pigs liver	99	71	71.7	9	0
Total	489	358	73.2	37	69

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Country report Campylobacteriosis in humans – Belgium

Akke Vellinga
Jean-Paul Butler

Incidence and trends

a. Human

Jaar	Incidence (/10^5inhabitants)	Number of cases	Average number of cases/lab/year
1993	44	4394	34,2
1994	48	4879	37,4
1995	47	4779	37,2
1996	49	4991	38,2
1997	55	5617	43,6
1998	65	6610	51,8
1999	64	6514	52,2

Food – *Evolution of food* Campylobacter *prevalence '97-'99*

		1997	**1998**	**1999**
Pork	Carcasses	16,3%	15,3%	21,4%
	Liver	28,3%	32,9%	
	Retail Cuts	2,6%	9,4%	12,5%
	Ground Meat	3,3%	6,2%	2,0%
Broilers	Carcasses	71,0%	72,6%	75,9%
	Liver	61,7%	74,6%	
	Breasts	81,8%	83,4%	57,6%
Layers	Carcasses	91,7%	82,3%	90,2%
Turkeys	Carcasses	72,5%	86,7%	
Beef	Carcasses	3,3%		
	Liver	31,7%		
	Retail Cuts	5,0%		
	Minced Meat	0,0%		
Veal	Carcasses	0,0%		
	Liver	11,7%		
	Minced Meat	0,0%		
Fish				2,3%
Rabbit		4,2%		

Surveillance

a. Human

Data were obtained from passive surveillance through reference laboratory results from patients with bloody diarrhoea. All cases are updated weekly by electronic mail.

b. Food

A screening programme was performed in 1999 (April to December) by the Veterinary Inspection Services (National Reference Laboratory for Food Microbiology, Ministry of Social Affairs, Public Health and Environment, Prof. G. Daube). More than 120 Belgian slaughterhouses and more than 100 meat cutting plans, representative of the Belgian production of carcasses and meat, were selected for this study.

	Species	Prevalence	Main Serotypes
Pork	Carcasses (N=154)	21,4%	Coli (79%) Lari (3%)
	Retail Cuts (N=152)	12,5%	Coli (44%) Jejuni (11%)
	Ground Meat (N=149)	2,0%	Jejuni/Coli (33%) Unknown (67%)
Broiler	Carcasses (N=141)	75,9%	Jejuni (70%) Coli (16%)
	Breasts (N=139)	57,5%	Jejuni (78%) Coli (14%)
Layer	Carcasses (N=122)	90,2%	Jejuni (70%) Coli (25%)
Fish	(N=131)	2,3%	Jejuni (33%) Coli (33%)

3. Food borne disease

Information on outbreaks (>2 cases) due to Campylobacter are reported obligatory to the French and the Flemish community. Suspected food items can be sent to the NRL to be examined (free service). A surveillance system for rapid alerts will be set up in the nearby future.

4. Control and prevention of Campylobacter

No national systems are set up to control or prevent Campylobacter.

Campylobacteriosis in the Netherlands

Arie Havelaar, Matty de Wit, Wilfrid van Pelt, Yvonne van Duynhoven, Nelly Voogt, Enne de Boer, Rob Willems, Birgita Duim, Wilma Jacobs-Reitsma,Jaap Wagenaar

Incidence in humans

General-practice based and community-based epidemiological studies

In the Netherlands, two studies have been performed to estimate the incidence of gastroenteritis and associated pathogens (bacteria, viruses and parasites); a study in the community in 1999 (Sensor), and a study among cases consulting a general practitioner in the period 1996-1999.

The incidence of gastroenteritis in the community was 283 per 1,000 person years. *Campylobacter* was isolated from 2.4% of cases, yielding an incidence of *Campylobacter* associated gastroenteritis in the community of 6.8 per 1,000 person years. In 1991, a population-based surveillance study on the incidence of acute gastroenteritis was performed, leading to an age-standardised estimate of 447 episodes per 1000 person-years. *Campylobacter* was isolated from 4.5% of faecal samples. Thus, both the incidence of gastro-enteritis and the percentage of *Campylobacter* positive faecal samples were higher in 1991. Most probably, the 1991 study has overestimated the incidence of campylobacteriosis because it was carried out in spring-early summer in a limited region of the Netherlands and used a different case-definition and symptom-reporting procedure.

The incidence of gastroenteritis for which a general practitioner was consulted was 8 per 1,000 person years. However, from comparing both studies we learned that under-ascertainment in the general practice-based study was substantial and after adjusting for this, we estimated an incidence of gastroenteritis in general practice of 14 to 35 per 1,000 person years. *Campylobacter* was isolated from 10.5% of cases in general practice, yielding an incidence of *Campylobacter* in general practice of 1.5 to 3.7 per 1,000 person years. No decreasing trend over the years was observed in the percentage attributable to *Campylobacter*, nor in the incidence of gastroenteritis in the GP-study between 1996-1999. In conclusion we can say that in the Netherlands, on a population of 15.76 million, annually 107,000 cases of campylobacteriosis occur, of which 23,000-58,000 consult a general practitioner, *i.e.* 22-54% of all cases in the population.

A similar study in 1992-3 found an incidence of 9 GP consultations for total gastro-enteritis per 1,000 pyr and *Campylobacter* was isolated from 14.6% of all faecal samples. Thus, against a stable background of GP visits, the prevalence of *Campylobacter* has decreased between these two studies.

Laboratory-based surveillance

15 regional public health laboratories report the total number of first isolates of Campylobacter spp. each week since April 1995. These laboratories effectively cover 62% of the Dutch population. Two laboratories (covering more than 1 million inhabitants) deliver their data electronically, supplying data on age, sex, residence and antibiotic resistance for the period 1996-1999. Between 1996 and 1999, *Campylobacter* was the main pathogen isolated in faecal samples (3.4%) with an average annual incidence of 36/100.000, *i.e.* 5700 laboratory confirmed cases of campylobacteriosis per year for the whole of the Netherlands. This is 5.3% of all cases in the population. The number of isolates of *Campylobacter* decreased significantly between 1997 and 1999 (15%). However, in 2000 up until November, it is 7% higher as compared to the same period in 1999. There is distinct seasonal variation in the incidence of campylobacteriosis. At the end of May isolations of *Campylobacter* strongly increase and peak in early September. In accordance with the GP-sentinel study and

findings elsewhere in developed countries the incidence is highest among the youngest children 0-4 years of age and higher as well in young adults 15-29. The incidence was 2-3 times lower in the age classes 5-14 and those 30 years and older. Quite a large effect was found related to the degree of urbanisation, incidences in rural areas being half of those found in urban regions.

Health burden

Infection with thermophilic *Campylobacter* spp. (mainly *C. jejuni*) usually leads to an episode of acute gastro-enteritis, which resolves within a few days to a few weeks. Occasionally, more severe and prolonged diseases may be induced, notably Guillain-Barré syndrome, reactive arthritis or bacteremia. For some patients, the disease may even be fatal. Data on the epidemiology of illness associated with thermophilic *Campylobacter* spp. in the Netherlands in the period 1990-1995 was integrated in one public health measure, the Disability Adjusted Life Year (DALY). DALYs are the sum of Years of Life Lost by premature mortality and Years Lived with Disability, weighed with a factor between 0 and 1 for the severity of the illness. There is considerable uncertainty and variability in the epidemiological information underlying the estimated health burden, which is explicitly taken into account in the analysis. The estimated health burden of illness associated with thermophilic *Campylobacter* spp. in the Dutch population is estimated by simulation as 1400 DALY per year (90% confidence interval 900-2000 DALY per year). The main determinants of health burden are acute gastro-enteritis in the general population (310,000 cases, 290 DALY), gastro-enteritis related mortality (30 cases, 410 DALY) and residual symptoms of Guillain-Barré syndrome (60 cases, 340 DALY).

Our prevalence in poultry and other food animals

In 1997, the National Institute of Public Health and the Environment started a surveillance program of zoonotic agents in farm animals on behalf of the Veterinary Public Health Inspectorate. This surveillance program is focused on VTEC O157, *Salmonella* spp. and *Campylobacter* spp. in faecal samples of poultry layer and broiler flocks, fattening pigs, veal calves and dairy cows. Approximately thousand farms are sampled each year. Sampling plans are based on statistical principles. Sixty fresh faecal samples from a flock are randomly collected and pooled into five pooled samples. A flock is considered positive for a certain pathogen, if one or more of the pooled samples is found positive. Samples are taken randomly during a cycle; hence the data are not directly representative for the prevalence of contamination at the end of the fattening round. Because contamination with *Campylobacter* usually becomes apparent after two to three weeks and gradually increases with time, the prevalence in flocks ready for slaughter will be higher than reported here. This becomes clear from survey results of flocks from a limited number of farms, of which the contamination levels were between 50 and 72% at the day of slaughter.

The survey will yield knowledge of the prevalence and trends of zoonotic agents in farm animal populations. Furthermore, epidemiological relationships between presence of pathogens in

Surveillance of Campylobacter *spp. in farm animals, the Netherlands, 1997-2000*

Category	Year	*Campylobacter* spp. (no of pos. flocks /no of flocks tested (%))	
Broilers	1997	21 / 47	(45)
(random)	1998	58 / 189	(31)
	1999	26 / 151	(17)
	2000[2]	25 / 97	(26)
(end of cycle)	1997	26 / 41	(64)
	1998	106 / 214	(50)
	1999	143 / 197	(72)
dairy cows	1997		n.t.[1]
(random)	1998	41 / 130	(31.5)
	1999	11 / 167	(6.6)
	2000		n.t.
veal calves	1997		n.t.
(random)	1998	52 / 62	(83.9)
	1999	35 / 60	(58.3)
	2000		n.t.
fattening pigs	1997		n.t.
(random)	1998	37 / 38	(97.4)
	1999	86 / 190	(45.3)
	2000		n.t.

[1]n.t. = not tested

[2]January - September 2000

farm animals and potential risk factors can be determined, as in the program also general information about the farm and its management is collected.

In November 1996, Government and the Product Boards agreed on the implementation of additional screening and hygiene measures in different levels of the poultry production chain. The plan was aimed at reducing the prevalence of *Salmonella* and *Campylobacter*. As the objectives of this plan were not met, labelling of contaminated products will be compulsory as of February 15, 2001.

In the surveillance study, the prevalence of *Campylobacter* in broilers decreased from 44.7% in 1997 to 17.1% in 1999. The reasons for this have not yet been evaluated. It may be related to sampling effects, but may also be related to the above mentioned action plan in the poultry sector. The year 2000 appears to give an increased prevalence, but this may in part be due to the fact that the year is not yet complete, and isolation rates in the fourth quarter are usually lower. Diary cows, veal calves and fattening pigs are also frequently contaminated with *Campylobacter* spp.

Prevalence in food

The Table below summarises the available information on the prevalence of *Campylobacter* in raw foods in the Netherlands, as investigated by the Keuringsdienst van Waren (Inspectorate for Health Protection and Veterinary Public Health). Samples were taken randomly from retail outlets, sales points for chicken products reflected sales volumes (e.g. more samples from supermarkets).

The data show that chicken products are still the most frequently contaminated type of food. *C. jejuni* is the most frequent species observed in poultry samples. In the past five years a slow but consistent decrease in prevalence of *Campylobacter* was observed, from 36.2% in 1996 to 23.5% in 1999. In 1994, shellfish (oysters and mussels) were also regularly contaminated with *Campylobacter*, *C. lari* being the most frequently isolated serotype. In 1999, no contaminated samples were observed, probably due to better hygiene at production sites and effective use of UV irradiation of process water. Beef, pork, fowl other than chicken and raw vegetables were infrequently contaminated with *Campylobacter*.

Multi locus sequence typing and AFLP typing of *Campylobacter jejuni*

75 *C. jejuni* strains from human (including strains from three outbreaks), animal and environmental origin from different countries were analysed by multi-locus sequence typing (MLST) of housekeeping genes and these results were compared with the results of AFLP fingerprinting analysis. Thirteen to 20 different alleles were identified resulting in 54 different sequence-types (STs). Sequence types were grouped into sequence lineages when alleles of four or more loci were identical. Assignment of the STs into lineages revealed that 10 STs were unique and 44 were assigned to 8 lineages. The majority of the isolates originating from humans and poultry belonged to the two largest ST lineages, 6 and 8. In lineage 6, 45% of the human and 57% of the poultry isolates were found, while in lineage 8, 25% of the human and 9% of the poultry isolates. This means that most (70%) of the strains recovered from humans grouped with a large subset (66%) of poultry isolates. Housekeeping gene sequences show that horizontal exchange of genetic information has a major influence on the structure and evolution of *C. jejuni* populations.

Resistance to antimicrobial agents

Resistance to fluoroquinolones in *Campylobacter* isolates from humans is increasing and is attributed to treatment of broilers with fluoroquinolones. In 1989 the prevalence of resistance to fluoroquinolones of *Campylobacter* from broilers was 14% and from humans 11%; in 1994 this amounted to 33% and 19% respectively.

Product	Year	Number of samples	Number (%) positive
Beef	1999	738	3 (0.4)
Pork	1999	524	0 (0.0)
Mixed pork/beef	1999	275	4 (1.5)
Chicken products	1996	1325	480 (36.2)
	1997	1314	417 (31.8)
	1998	1076	282 (26.2)
	1999	859	202 (23.5)
Turkey	1999	145	1 (0.7)
Duck (wild)	1999	52	3 (5.8)
Pheasant	1999	27	1 (3.7)
Guinea-fowl	1999	35	1 (2.9)
Shellfish	1994	100	52 (52.0)
	1999	97	0 (0.0)
Vegetables (raw)	1999	966	3 (0.3)

Between 1995 and 1999 the prevalence stabilised at 21% in humans. Treatment amounted to 17% of the broiler flocks in 1999. Differences between groups of patients with campylobacteriosis with respect to the level of resistance to fluoroquinolones would therefore reflect differences in exposure to *Campylobacter* infected poultry meat. Between 1996 and 1999 human campylobacteriosis was 3-6 times more frequent in the summer as in the winter whilst resistance to fluoroquinolones was found to be 3-6 times lower in the summer. Amongst humans living in rural areas the Campylobacter incidence is half that found in urban areas whilst *Campylobacter* resistance to fluoroquinolones is two-third of that found in urban areas. Furthermore, *Campylobacter* resistance to fluoroquinolones amongst adults 20 years and older is higher than amongst children, gradually decreasing towards two-third of the adult level amongst the youngest children.

The tentative conclusion is that in people infected in the summer months, in young children or in people living in rural areas a clearly larger fraction of the *Campylobacter* infections originates from other sources than poultry. Nothing is known however of the species distribution. Knowing the level of resistance of *Campylobacter* in broilers from week to week in parallel to that in humans allows the computation of the approximate fraction of human campylobacteriosis associated with poultry. To this end, since May 2000, *Campylobacter* resistance and species distribution is monitored in almost 25% of all broilers slaughtered.

Conclusions

Thermophilic *Campylobacter* species are the most frequently identified bacterial agents of gastroenteritis in the Netherlands. The health burden associated with campylobacteriosis is considerable, also due to association with Guillain-Barré syndrome and reactive arthritis. It is difficult to obtain an unbiased estimate of the true incidence of campylobacteriosis in the general population or among consultations to general practitioners. It is even more difficult to obtain a representative picture of the duration, severity and impacts of campylobacteriosis in the general population. Due to methodological difficulties it is not possible to draw conclusions on trends from comparing point estimates of incidence by cohort studies. Laboratory surveillance appears to be more useful for trend evaluations, but identifies only a minor part of all cases. If anything, there has been a decreasing trend in incidence/ prevalence of *Campylobacter* in humans, as well as in poultry (broilers) and poultry products in the Netherlands in the nineteen-nineties. Nevertheless, the incidence of human campylobacteriosis is still high, and additional measures are required to obtain a further reduction. MLST and AFLP typing are emerging as promising tools for establishing genetic relationships of strains from different sources.

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Campylobacter infections in France (1986-1997)

Francis Mégraud

Sources: National Reference Center and referring microbiology laboratories

Overview prepared by the National Reference Center for Campylobacters and Helicobacters, Laboratoire de Bactériologie, CHU Pellegrin, Bordeaux, France

1. Objectives, modalities and quality of the surveillance system

1.1. Objectives

To determine the epidemiological characteristics of Campylobacter infections, to identify the species involved, and to follow the rate of antibiotic resistance, in particular, to quinolones.

1.2. Modalities

The surveillance is based on a network of hospital (general hospitals and University hospitals) laboratories located nationwide who send Campylobacter isolates to the National Reference Center (NRC) along with corresponding epidemiological information. The network has been operating since 1986. However, during the past few years the strains isolated from mild diarrheal episodes, particularly in children, have no longer been sent, as they correspond essentially to *C. jejuni* strains whose characteristics have remained stable over this period.

Furthermore, the Center receives strains from other laboratories, isolated for the most part in a context of severe infection, and for which assistance in identification is needed.

1.3. Qualities

The network system is far from being exhaustive and is not representative of the situation of Campylobacter infection in the community. Nonetheless, it constitutes an imperfect reflection on Campylobacter infection in French hospitals.

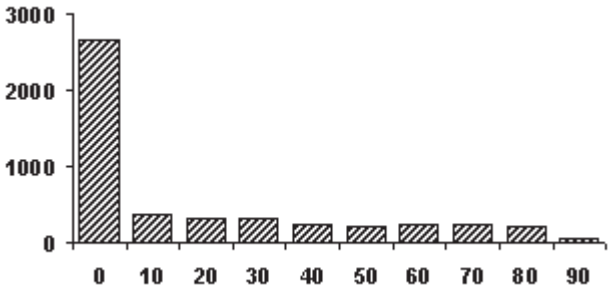
2. Main epidemiological characteristics

The data presented corresponds to 5,707 strains isolated between 1986 and 1997, of which 3,988 were studied at the NRC.

2.1. Distribution according to age

A large number of strains were isolated from children under the age of 10. The subsequent numbers per decade are relatively stable.

Figure 1. Number of Campylobacter strains classified by the NRC according to age, from 1986 to 1997.



2.2. Distribution according to gender

There is a clear predominance of Campylobacter infections in males (60%).

2.3. Distribution according to the month of isolation

There is a seasonal peak of Campylobacter isolation during the summer months, with the greatest number found in August (fig. 2).

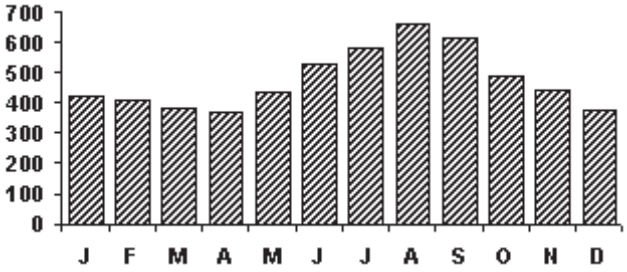
2.4. Origin of the contamination

A family outbreak was observed in 7% of the cases. However, this information was only available for 1,926 of the isolated strains (33.7%). A contamination acquired abroad was noted for 14% of the isolates.

Table 1. Distribution of the different Campylobacter species isolates according to the type of specimen tested, for the strains received at the NRC which were able to be subcultured (France, 1986 to 1997).

Strain	Faeces	Hemoculture	Other specimens	Not known	Total
C. jejuni	2554	109	38	12	2713
C. coli	714	36	12	7	769
C. fetus	67	198	45	15	325
C. upsaliensis	29	2	2		33
C. lari	18	5	3		26
C. sputorum	3	1	2		6
C. hyointestinalis	3				3
A. cryaerophila	5		2		7
H. cinaedi	1	3			4
Campylobacter sp.	75	17	9	1	102
Total	3469	371	113	35	**3988**

Figure 2. Distribution of Campylobacter strains received at the NRC according to the month of isolation, from 1986 to 1997.



2.5. Distribution of the different Campylobacter species isolated according to the type of specimen (Table 1)

C. jejuni was by far the species most frequently found (68% of the isolates) and almost all of them were isolated from fecal specimens. *C. col*i was isolated in 19% of the specimens, and *C. fetu*s in 8%. The two other species which were encountered were *C. upsaliensis* and *C. lari*. It should be noted that *C. fetus* is often at the origin of septicemia and secondary localizations, however, regarding the total number of these cases, there are almost as many due to thermophilic Campylobacters (*C. jejuni* and *C. col*i) as to *C. fetus*.

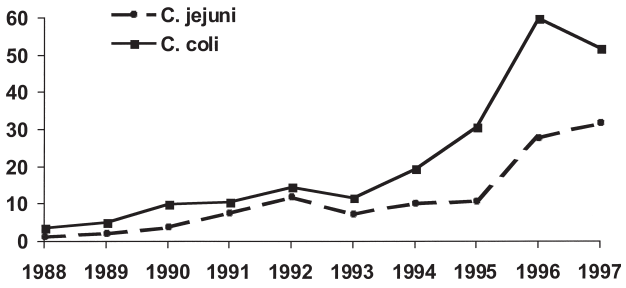
2.6. Evolution of antibiotic resistance

The antibiotic susceptibility of Campylobacters was evaluated by the NRC using the disk diffusion method. Since 1986, the antibiotic resistance of Campylobacters, with the exception of quinolones, has remained stable but at different levels depending upon the antibiotic (Table 2). The resistance rates to macrolides and to tetracycline are very low. No strain was resistant to gentamicin. In contrast, an alarming evolution of quinolone resistance has been observed these last few years for *C. jejuni* and even more so for *C. coli* (fig. 3): from 1993 to 1997 the proportion of resistant strains increased from 7.4 to 32% for *C. jejuni* and from 11.8 to 52% for *C. coli*. Comparable evolutions regarding quinolone resistance have been found in other countries, although the type of referral of strains to be studied by the NRC may have influenced the evolution of the resistance rate.

Table 2. Campylobacter resistance to antibiotics (3,988 strains tested at the NRC from 1986 to 1997)

Antibiotic	Proportion of resistant strains (%)
Erythromycin	3.6
Tetracycline	9.5
Streptomycin	7.1
Gentamicin	0.0
Kanamycin	1.0
Ampicillin	50.0

Figure 3. Evolution of C. jejuni and C. coli resistance rates against quinolones from 1988 to 1997.



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Department of Communicable Disease Surveillance and Response