

**UK PUBLICLY-FUNDED RESEARCH RELATING
TO *CAMPYLOBACTER***

**Report of the
Microbiological Safety of Food Funders Group
(MSFFG)**

INTRODUCTION

1.1 The primary aim of this paper is to provide an overview of publicly funded research relating to *Campylobacter*. The details relating to funded projects have been provided by Members of the Microbiological Safety of Food Funders Group (MSFFG) who have considered the available information in order to make an assessment of the findings **up to the end of June 1999** and identify any gaps in funded research. In making its assessment the MSFFG has considered the views and recommendations of expert Groups such as the Advisory Committee on the Microbiological Safety of Food (ACMSF) and the World Health Organisation (WHO). The MSFFG has defined gaps in research as “perceived lack of information”. Such gaps in research will be considered as possible priorities for additional work, but will not necessarily be addressed by Government funding.

1.2 The aim of this work is to also identify overlaps in publicly funded research to ensure a complementary and coherent research programme. In some instances work has been funded with similar titles, but examination of the ongoing work has revealed complementary rather than overlapping objectives.

1.3 It is not the aim of this paper to devise a strategy of control for *Campylobacter* infection, but the information presented will be used to inform a strategy for control.

1.4 In some instances the details of the current state of publicly funded research projects have not been included. This is where a project has only recently started and no details are yet available. The paper has considered publicly funded research **from 1990 to the end of June 1999**. Table A1.3 lists new research that has started since July 1999 and this will be updated on a regular basis.

1.5 The information provided in this paper has been provided by members of the MSFFG or by their contractors. The findings presented have not necessarily been peer reviewed, although all contractors are encouraged to publish the findings of their research in peer reviewed literature.

1.6 The information on funded research has been considered under the following headings:

- Detection, differentiation and diagnosis
- Microbial physiology and genetics
- Pathogenesis
- Epidemiology
- Surveillance
- Risk analysis
- Reduction and elimination
- Microbial antibiotic resistance
- Other

Although the projects assessed have been considered under these defined categories, a lot of the research has been funded with multiple purpose, i.e. covering more than one

research area. The paper does not aim to cover research associated with *Campylobacter* in water or direct contact with *Campylobacter*, but research in these areas which has implications for the microbiological safety of food has been referred to.

1.7 This overview paper on publicly funded research on *Campylobacter* represents one of the outputs of the deliberations of the MSFFG and the format will be repeated for other foodborne pathogens. These overview papers address the Group's terms of reference "*To assist the co-ordination of publicly funded research and development on the microbiological safety of the food chain with a view to informing the R&D effort, identifying gaps and overlaps, and to provide reports as appropriate*".

2. DETECTION, DIFFERENTIATION AND DIAGNOSIS

Introduction

2.1 The ACMSF (1) made a number of recommendations for Government funded research in the area of laboratory methods for the detection and sub-typing of *Campylobacter* spp.. It was recommended that research be undertaken into:

- isolation and identification methods that can be used by clinical laboratories for the detection of all clinically relevant *Campylobacter* species.
- sub-typing methods which will enable better epidemiological tracing of sources and transmission routes of human infection.
- better detection methods for viable non-culturable forms of *Campylobacter* in order to determine whether they play a role in the production of human enteritis.
- establishment of whether all strains of *C.jejuni/coli* from whatever source have equal disease causing potential for humans.

2.2 The need for better methods of *Campylobacter* subtyping to help clarify sources and transmission routes was recommended in the ACMSF's Report on poultry meat (4). In addition, following a review of *Campylobacter* research held during November 1996, the Committee expressed concern that there was no standard subtyping method for *Campylobacter* and recommended the Government bring researchers together to work towards a common method.

2.3 The WHO (2) recommended the urgent need for collaborative research, on an international basis, on techniques for the isolation of campylobacters from food and environmental samples. The WHO noted that this is essential for the proper conduct of epidemiological and intervention studies and recommended the setting up of a WHO Working Group to discuss this matter and to co-ordinate future activity.

2.4 The WHO recommended research into improved methods for speciation and for sub-typing of *Campylobacter* and that such methods need to be internationally standardized. It was recommended that the WHO should assist in establishing a working group to advise and recommend appropriate techniques.

2.5 The detection, differentiation and diagnosis category holds the largest number of projects for any one area (thirty-one). Within this classification lie projects more principally related to a variety of other areas such as epidemiology, pathogenesis, microbial physiology/genetics and surveillance. However, the largest group of projects relates to sub-typing.

Current State of Publicly Funded Research

Detection

2.6 The efficacy of published methods for the isolation of *Campylobacter* from contaminated surfaces has been investigated (DH, 176). The work demonstrated that *C.jejuni* survives well in moist droplets (blood) but when dried the organism can not be isolated. For surfaces contaminated with raw chicken, isolation was improved by placing swabs directly into enrichment broth containing the highly selective Exeter antibiotic formulation. In general terms, isolation was better using Exeter broth compared to others commonly used: Exeter>Preston>laboratory medium>Parker & Saunders. For transport media or diluents, the use of fastidious anaerobe broth (FAB) was superior to buffered peptone water and also enhanced survival of damaged *C.jejuni* cells. The findings indicate that isolation of campylobacters from catering surfaces can be improved if sampling is concentrated on moist areas and if swabs are placed directly into Exeter selective broth.

2.7 A collaborative trial was undertaken to compare the DIS (Park and Sanders) method, Exeter and ISO method for the detection of thermophilic *Campylobacter* spp.. It was recommended that the DIS method continue to be used for MAFF microbiological food surveillance (MAFF, FS1254).

2.8 As part of a pilot study for a survey of *Salmonella* and *Campylobacter* in poultry on retail sale, three different sampling methods were evaluated, neck-skin, rinse and rinse+skin samples (DH, 271). Similar results were obtained using the three sampling methods with rinse+skin giving the highest detection rates for *Campylobacter* spp. (isolation rates for neck-skin, rinse and rinse+skin samples were 88%, 90% and 93% respectively).

2.9 In a project investigating viable non-culturable organisms, it was found that under some conditions plate counts can underestimate the viable count by a 100 fold. However, because the number of non-culturable cells represents a small fraction of the total cell population, it was not possible to establish by direct observation whether the 100-fold increase caused by resuscitation represented repair of injured vibroid cells or reversion of coccoid forms (MAFF, FS1525).

2.10 In a project aimed at investigating the physiological mechanisms for the adaptation of *Campylobacter* to stress injury, an immunological reagent was developed for the detection of novel proteins expressed during nutritional deprivation (MAFF, FS1526).

2.11 Several projects in the area of detection have aimed to produce a PCR based method for rapid screening of samples. An early project developed a sensitive and specific PCR for the detection of *C.jejuni* in foodstuffs and found a competitive interaction between calcium and magnesium ions which caused inhibition of PCR (MAFF, FS1214). In another project 16S RNA gene sequences were used to design

PCR assays specific for the genus *Campylobacter* and each of five species important in veterinary and/or human medicine (*C.upsaliensis*, *C.helveticus*, *C.fetus*, *C.hyointestinalis* and *C.lari*). The assay was able to rapidly identify species from faecal samples up to two weeks old (**DH, 220**). The assay has enabled a large-scale survey of *Campylobacter* species in human gastroenteritis to be carried out (**DH, 220B**, see section 2.28). The latest work being undertaken is based on the LightcyclerTM, an advanced PCR platform with the potential for quantitative detection of PCR targets as well as rapid and sensitive qualitative detection (**DH, 220C**).

2.12 A different approach was taken by another group who developed three PCR assays based on the *gly A*, *fla A* genes and an unidentified open reading frame (ORF-C) of *C. jejuni*. The three primer sets were incorporated into RT-PCR assays for the detection of mRNA from thermophilic *Campylobacter* spp.. A PCR-ELISA was also developed and shown to be as sensitive and specific as conventional culture based methods (**MAFF, FS1242**).

2.13 In a project aimed at defining the role of toxins in *Campylobacter* pathogenesis, PCR based methods were developed to detect specific genes, based on primers designed to produce internal fragments to the putative toxin gene and other virulence determinants (**DH, 112**).

2.14 Other approaches include the adaptation of phage amplification technology in order to detect *Campylobacter* spp. in food (**DH, 222**) and the development of a molecular and biosensor based generic technology capable of automation (**SERAD, URG/001/96**).

Differentiation

2.15 Several approaches have been taken to the issue of sub-typing of *Campylobacter* species. An early project evaluated the usefulness of rapid molecular based methods and four nucleic acid amplification methods based on the PCR reaction were developed (**MAFF, FS1217**). A second project devised a novel intraspecific molecular typing scheme which exploits the sequence conservation of *Campylobacter* genes encoding bacterial methyl-accepting chemotaxis proteins (**DH, 223**). It was demonstrated that a DNA probe comprising the Highly Conserved Domain region of the *t/pA* can be used in Southern Hybridisation based assays to provide novel information, allowing the discrimination of individual strains of *C. coli* and *C. jejuni*.

2.16 Later projects have sought to compare typing methods. It was found that PFGE and PCR finger printing were more discriminatory than ribotyping for *C. jejuni* and were reproducible between laboratories (**SODoH, K/MRS/50/C2192**). Ribotyping provided the same level of discrimination as Penner serotyping. Neither PFGE nor ribotyping were found to be suitable for speciation within the *Campylobacter* genus. However, sequencing of the 16S RNA gene allowed identification of every isolate tested.

2.17 The stability of PCR-RFLP typing of flagellin is being compared with RAPD and PFGE typing methods in conjunction with European partners in the EC funded

CAMPYNET programme. Speciation by PCR-RFLP of the RNA genes has proved successful to date and species specific probes are being developed based RAPD profiles (**DANI, 9461**).

2.18 Work to determine which method or combination of methods would be appropriate for typing of *Campylobacter* in surveillance and outbreak investigation has evaluated phenotypic and genotypic methods (**DH, 159**). About 2400 isolates of *Campylobacter* were collected, mainly in the UK, from human infections (sporadic and outbreak-associated cases), as well as from a variety of animal, avian, food and environmental sources. Also relevant reference strains were examined. Ten typing/subtyping methods were assessed on a panel of selected performance and convenience criteria using the strains. The methods were:

- biotyping
- resistotyping
- serotyping with heat stable antigens (Penner scheme)
- phage typing
- ribotyping
- pulsed field gel electrophoretic (PFGE) profiling
- flagellin gene profiling
- repeat sequence / AP-PCR profiling
- hippuricase gene profiling
- *rrn* intergenic (spacer) region profiling

2.19 The researchers found that no single typing method satisfied all criteria. PFGE profiling provided the highest level of discrimination but lacked the speed and high throughput capacity of phage typing. Phage typing needs further methodological development, and both methods need standardisation before they can be accepted as general purpose schemes. Resistotyping and other new PCR based molecular methods (Fla-typing and REP/AP-PCR typing) were mostly not serotype specific and appeared to have limited potential for development into new general purpose typing schemes, although they provided some strain markers of value in outbreak investigation. Ribotyping provided fundamental genomic information about strain associations but was labour intensive and consequently of limited practical value for routine typing. The other methods studied (biotyping, hippuricase gene and *rrn* spacer region profiling) gave only low discrimination between strains and were of limited use for most typing applications.

2.20 The researchers concluded that at present there is no wholly satisfactory approach to typing of campylobacters. For reference surveillance and outbreak investigation the use of HS serotyping in combination with phage typing and/or PFGE profiling would provide a high level of typability and discrimination. Resistotyping is the only feasible possibility for local outbreak investigation but such data is not definitive and would need to be supported by subsequent detailed reference typing.

2.21 The Department of Health also funded a collaborative group of workers to investigate methods for sub-typing *C.jejuni* (**DH 159A-159E**). The study showed that the most successful methods for recognising outbreak clusters were sero-typing (Penner),

bio-typing by disc susceptibility resistotyping and pulse field gel electrophoresis (PFGE). The most discriminating methods based on the selection of the strain used were PFGE, random primer profiling by PCR (RAPD), PCR typing of flagellin gene (*Fla A*) and multi-locus enzyme electrophoresis typing (MEE). Non-typeable strains were encountered by sero-typing, phage-typing and PFGE.

2.22 The researchers concluded that no single method is suitable for definitive sub-typing of *C.jejuni* and epidemiological investigations would require a combination of typing techniques. Penner typing should form the basis of a primary sub-typing scheme for *C.jejuni*. PFGE, *Fla A* PCR gene typing, ribotyping, and phage typing were considered to be suitable techniques for *C. jejuni* sub-typing depending on the situation i.e. reference or local needs. In addition, RAPD and biotyping were considered to be potentially suitable for local surveillance studies.

2.23 Efforts are being made to co-ordinate the typing of veterinary, food and human isolates. The PHLS serotyping scheme has been established at VLA and is being used to type isolates from poultry. However 40% of the poultry isolates were found to be untypable and this is being investigated in more detail in collaboration with PHLS. VLA are looking into the extent of the non-typability, the effect of isolation, growth and storage conditions and are trying to identify common phenotypic and genotypic properties in non-typable strains (**MAFF OZ0601**).

2.24 A comparison of PHLS serotypes and Penner serotypes showed that correlation between the two was weak given that the same reference strains were used to produce the typing sera. A comparison of the PHLS serotypes with *Fla* types showed that for each *Fla* type the majority of typable strains comprised of a single serotype, indicating corresponding clonality independently identifiable in both systems. However, both schemes could further differentiate individual types from the other scheme (**MAFF, OZ0140**).

2.25 Two projects have investigated the prevalence and distribution of *Campylobacter* in poultry processing plants, using a variety of typing techniques (**DH, 227 and MAFF, FS1052**). It was found that improved methods are required to detect low numbers of clinically significant strains within a mixed population of the same organism. The most prevalent strains were found to be those which were difficult to serotype.

2.26 Penner serotyping, *flaA* typing and PFGE were used to establish whether the re-use of contaminated transport crates made a significant contribution to broiler carcass contamination. Typing results suggested that the crates were a source of contamination, either directly or via the handlers' hands, but numbers on the birds were low (**DH, 227B**).

2.27 Several projects have looked at variation amongst campylobacters. In a study to examine microbial adaptation to the environment and survival in the food chain, genetic typing techniques (such as AFLP) will be used to examine the population structure of pathogens in the natural environment (**BBSRC, 3258371**). The genotypic and phenotypic instability of 200 outbreak related and non-outbreak related strains is also

being studied to determine the mechanisms underlying genotypic variation (**MAFF, OZO605**).

2.28 To establish the range and nature of *C. jejuni* isolates, genetic and antigenic variation is being assessed in order to produce a database cataloguing variability and to develop models of population biology. Analysis of the data aims to determine the factors involved in genetic variation (**MAFF, OZO604**). In a study to trace the spread of campylobacters from the farm environment, flagellin gene typing and PFGE are being used to examine the genetic diversity of isolates from clinical, animal and environmental sources. A wide range of genotypes were found with several common to both animals and humans. Conversely some were host specific (**DH, 221**).

2.29 A project investigating beef cattle faeces at slaughter in NI abattoirs found that 25.7% of samples were positive for *Campylobacter* (n = 210). PFGE showed some similarity between isolates from specific abattoirs but in general a diversity of types was observed with no one type dominating (**DANI, 9723**).

Diagnosis

2.30 Two projects have been funded in the area of diagnosis. The first was a study of the prevalence of *Campylobacter* in human disease and used a recently developed PCR-ELISA assay. The assay has enabled a large-scale survey of *Campylobacter* species in human gastroenteritis to be carried out (**DH, 220B**). Faecal samples (3,738) from patients with sporadic cases of acute gastroenteritis were studied over a two year period. While there was no statistically significant difference between PCR and culture in detection of *C. jejuni/C. coli* (PCR: 478 samples; culture 461 samples), PCR provided unique data about mixed infections and non-*C.jejuni/C.coli* campylobacters (see section 5.8 for more details).

2.31 The second project developed an immunological assay which could be used to determine the prevalence of potentially immunologically protected or susceptible individuals. Chicken abattoir workers and those who had been diagnosed as having had a recent infection were sampled for serum, saliva and urine. The results indicated that a test for long term immunity to *C.jejuni*, measuring serum or salivary IgG antibodies is feasible and that flagellin, MOMP and a 40kD antigen may be important components of any test developed (**DH, 186**).

MSFFG Assessment and Identification of Gaps

2.32 In the area of detection there has been a lot of progress in the development of rapid molecular detection methods. However, the following gaps remain:

- The area of communication between researchers to ensure that validated molecular methods are harmonised and standardised for the detection of contaminated food, water and animals

- The harmonisation and standardisation of sampling and culture techniques for human, animal and environmental samples
- The development of a rapid method for the detection of contaminated poultry flocks at or before slaughter

2.33 In the area of differentiation there is a need for communication between researchers to ensure that subtyping methods are well defined so that internationally acceptable typing schemes can be established.

2.34 There is currently no unified approach to *Campylobacter* subtyping. A number of research groups have investigated a range of available subtyping methods and recommended the use of Penner serotyping as a primary sub-typing scheme for *Campylobacter*. Depending on the situation (i.e. local or reference needs) suitable techniques for further subtyping include PFGE, phage typing and resistotyping. There remains a gap in communicating this important message to the research community so that these methods can be introduced as the standard approach to *Campylobacter* subtyping.

2.35 With respect to diagnosis of human disease, there is a gap in our knowledge for non-invasive diagnostic tests, e.g. salivary based tests.

3. MICROBIAL PHYSIOLOGY AND GENETICS

Introduction

3.1 The ACMSF (1) made a recommendation for Government funded research in the area of microbial physiology and genetics of *Campylobacter* spp. relating to viable non-culturable forms of *Campylobacter* in order to determine whether they play a role in the production of human enteritis. A recommendation was also made to industry to fund the effect of preservatives and use of modified atmospheres in processing and packaging on the survival or inactivation of campylobacters in food.

3.2 Sixteen projects fall within the category of microbial physiology and genetics. The research covers the areas of survival and environmental adaptation, metabolism and genetics.

Current State of Publicly Funded Research

Survival and environmental adaptation

3.3 Several projects have looked at the adaptation of campylobacters to stress injury. One study has demonstrated that both *C. jejuni* and *C. coli* are able to grow aerobically on agar plates but only in atmospheres of high humidity and that superoxide dismutases plays a role in the bacterial defence against the stresses imposed during freezing **(MAFF, FS1509)**.

3.4 An integrated research programme has begun which will address how foodborne pathogens, including *Campylobacter*, adapt to and survive the stresses encountered during the food chain and how we can overcome enhanced resistance to ensure inactivation or inhibition of microorganisms in foods using the minimum treatment necessary **(BBSRC, 3258371)**. The population structure of pathogens that occur in the natural environment will be examined to determine whether strains causing foodborne illness in humans are the same or different from those that occur in animal reservoirs and whether there are significant differences in resistance and virulence properties between strains. The food-relevant aspects of a pathogen's cell cycle control and its response to environmental conditions will be described in molecular detail. When data on genetic strain differences become available the extent of selective advantage of particular strains will be quantified by modelling the behaviour of populations subject to food chain selective pressures.

3.5 A project is underway to investigate the responses to oxygen of campylobacters and thus provide underpinning knowledge for developing strategies to reduce *Campylobacter* survival in foods and during food-processing **(BBSRC/SERAD, BFP11346)**. A studentship project has also investigated cold adaptation and culturability in *C.jejuni* **(BBSRC, 96/B1/D/02408)**.

3.6 Adaptation to the environmental conditions found in the gut will be investigated in a project which focuses on the role of the RacR regulatory system (**BBSRC, D09207**). RacR mutants have an altered pattern of growth both at 37°C and 42°C and a reduced ability to colonise the chicken intestine. The regulatory system will be further characterised and its role in the physiology (and virulence) of campylobacters determined.

3.7 The role of non-culturable forms in survival in foods has been investigated (**DH, 132A**). The research demonstrated that nutrient stress greatly reduces survival, culturability and infectivity of campylobacters. The type of stress and the way the organism has been handled prior to culture influences this reduction in culturability, etc. The techniques developed and used to define the viable non-culturable (VNC) state showed that the ability of campylobacters to produce VNC forms is dependent on the strain and its exposure to previous culture and storage conditions. The VNC forms produced were unable to colonise one-day-old chicks. The protein and antigen profiles of campylobacters exposed to water environments demonstrated that campylobacters do adapt to the environment, both by up- and down- regulation of gene expression (**MAFF, FS1526**). Few novel proteins were expressed during nutritional deprivation, though some antigens were lost.

3.8 Continuous culture techniques have been used to identify the environmental conditions that trigger the switch from the spiral to the coccal form of *Campylobacter* cells, the latter associated with the VNC state (**DH, 132B**). High dissolved oxygen tension ($\geq 100\%$) was the only environmental factor that reproducibly produced the coccal morphology (50% enriched). Such a high dissolved oxygen tension, however, also dramatically reduced the growth rate and biomass of the culture so it was impossible to maintain continuously growing coccal cultures. Other environmental factors such as low pH, high pH, or iron limitation did not elicit coccal cell formation. The research shows that the single most important factor in coccal cell formation is high oxygen tension.

3.9 A project to study the recovery of full viability in dormant non-culturable cells of *Campylobacter* has shown that anaerobiosis gives rise to structurally dense cocci that appear more “alive” than those formed aerobically (**MAFF, FS1525**).

3.10 Another project investigating the survival of campylobacters (**DH, 168**) has shown that:

- *Campylobacter* strains differ markedly in their survival in water, by up to at least 5-fold, depending on temperature and oxygenation
- survival is promoted by temperatures in the range 4°C to 10°C and may persist in excess of a week
- survival is reduced to 1-2 days at temperatures of 22°C and above
- the influence of anaerobiosis varies considerably between isolates
- survival as determined by culture, was improved by the presence of autochthonous water flora and/or incorporation into aquatic biofilms
- persistence increased under these conditions, in excess of two to four weeks
- survival determined by IFA staining demonstrated that the persistence of the pathogen up to termination of the experiments (28 and 42 days) increased carbon

load in the water and increased biofilm production, but reduced *Campylobacter* survival.

Metabolism

3.11 Little is known about the metabolism of *C.jejuni*, in particular, the organisation of central carbon metabolism, the physiological basis for its microaerophilic nature and requirements for elevated levels of carbon dioxide for growth. The genome sequence of *C. jejuni* 11168 suggests a metabolic versatility, with evidence for several potential pathways for glucose catabolism, a complete citric-acid cycle and a highly branched electron transport chain. A project is underway to investigate key features of *C. jejuni* metabolism suggested by this genome sequence (**BBSRC/SERAD, BFP11294**).

Genetics

3.12 Work has recently started on a project which aims to construct an inexpensive DNA microarray of the entire *C. jejuni* genome for transcriptome analysis. This microarray will be used to quantitate mRNA expression of all genes in response to given stimuli. (**BBSRC/SERAD, BFP11362**). A collaborative project is also underway to identify protein expressed both under standard conditions and with selected stresses and genetic changes (**BBSRC/SERAD, BFP11390/BFP11391/BFP11392**).

3.13 Another project is underway to determine the natural occurrence of genetic and phenotypic instability of *Campylobacter* from environmental, animal and human sources and to determine the mechanisms underlying *Campylobacter* genotypic variation (**MAFF, OZ0605**).

MSFFG Assessment and Identification of Gaps

3.14 Gaps in our knowledge relating to the physiology of *Campylobacter* (in terms of the effect of temperature, pH, and water activity) are currently being addressed through funded research. However, there remains a lack of understanding of:

- The organism's metabolism, the physiological basis for its microaerophilic nature and its requirement for elevated levels of carbon dioxide for growth. Researchers should be encouraged to make the best use of the data arising from the *Campylobacter* genome project when investigating the organism's physiology and metabolism.
- The VNC state in *Campylobacter* - this appears to be a response to certain environmental conditions but it remains unclear whether VNC's are infectious for humans

4. PATHOGENESIS

Introduction

4.1 The ACMSF (1) made a number of recommendations for Government funded research in the area of pathogenesis of *Campylobacter* spp. It was recommended that research be undertaken to establish:

- why campylobacters cause disease in humans and in some animals but not in other animals.
- whether all strains of *C.jejuni/coli* from whatever source have equal disease causing potential for humans.
- the disease causing potential of the most recently described species: *C.lari*, *C.hyointestinalis* and *C.upsaliensis*.
- the role of toxin-producing strains in the UK.
- the role of viable non-culturable forms of *Campylobacter* in the production of human enteritis.

4.2 The WHO (2) recommended that research is needed to elucidate the mechanism of *Campylobacter* colonisation and the basis of resistance to colonisation in different lines of broiler chicken.

4.3 The Danish Veterinary and Food Administration (3) stated that information on the virulence of the various types of *Campylobacter* including the incidence and significance of the “viable but not culturable” form, dose-response relations, the exact incidence in the population and the degree and number of late reactions is not optimal.

4.4 Fourteen projects fall within the category of pathogenesis. The research covers the areas of virulence factors, adhesion and colonisation, and the development of an animal model of *Campylobacter* infection relevant to the human disease.

Current State of Publicly Funded Research

Virulence factors

4.5 A number of approaches have been undertaken to identify and characterise virulence factors in *Campylobacter*. One approach has been to determine the range of virulence properties of representative strains from human, veterinary, and environmental sources using assays for invasion and toxin production (**MAFF, OZ0602**). One of the aims of the study will be to provide estimates of the pathogenicity of *Campylobacter* strains from veterinary sources.

4.6 Work is underway to compare *Campylobacter* subtypes isolated from humans and from poultry and to identify subtypes unique to their respective hosts (**BBSRC, 3250762**). The aim is to utilise these host specific differences to identify potential virulence determinants in *Campylobacter*. Once identified these features would allow

more accurate assessment of the virulence potential and risk to humans of campylobacters derived from food sources. Another study investigating the variation in the virulence of *C.jejuni* strains associated with poultry and poultry meat has demonstrated considerable variation in the invasiveness of over 50 isolates (**MAFF, FS3107**). One *C.jejuni* strain, isolated from a small pool of water outside a broiler house exhibited “hyperinvasiveness”, i.e. 0.5% of the strain population invaded compared to 0.001% in other strains. Findings to date show that that growth, temperature and exposure to conditions mimicking those during the abattoir process (i.e. heat shock at 52°C for 1 minute, allowed to cool for 75 minutes and then stored at 4°C overnight) all significantly affected invasiveness.

4.7 Another approach has been to isolate and characterise genes encoding virulence determinants in *Campylobacter* (**DH, 223**). The aim of the work was to determine the prevalence of virulence traits in *Campylobacter* species (*C.jejuni*, *C.coli*, *C.fetus*, *C.lari*, *C.upsaliensis* and *C.hyointestinalis*) using molecular detection methods and determine their role in the disease process. An iron acquisition system, which is likely to play a role in virulence, has been identified in a number of *Campylobacter* species. In addition a protein (phospholipase A) has been identified which is responsible for cell associated haemolytic activity in *C.coli* and may represent a novel *Campylobacter* virulence determinant. *In vitro* models for invasion and cytotoxicity have been used, but its role in virulence has not yet been established. Nevertheless, the work has demonstrated a correlation between haemolytic activity and the possession of phospholipase activity in different strains and species of *Campylobacter*. Studentship projects are also underway to characterise the iron regulon and to characterise and investigate the biological role of the outer membrane proteins of *C.jejuni* (**BBSRC, 98/A1/P/04261 & 98/B2/D/04041**).

4.8 Other work has attempted to define the role toxins play in *Campylobacter* pathogenesis. One approach has been to identify the factors which modify toxin expression at a genetic level and to develop probes to detect such toxins (**DH, 112**). Previous research has suggested the involvement of a cholera toxin (CT)-like enterotoxin and a shiga toxin (SLT)-like cytotoxin in pathogenesis. Probes were designed to conserved regions of the CT enterotoxin and SLT cytotoxin genes and used to detect similar genes in *Campylobacter*. An SLT-like gene was detected and was partially characterised. Due to the high toxicity of SLT-like protein (which caused problems with *E.coli* host/vector systems) full characterisation of the protein was not completed. However the results suggest that, like SLTs, the protein may be inhibitory to ribosomes. No CT-like genes were detected. Cytotoxic activity has also been investigated in another study that investigated the importance of cytotoxin production by characterised strains from humans, cattle and chickens (**DH, 228** – see section 4.11 for additional information on this project). The cytotoxicity assay, based on inhibition of the ability of active mitochondria to reduce a tetrazolium dye, showed that none of the assays achieved 100% killing of the test cells (CaCo-2, Vero and HeLa cells), indicating that the toxin activity was not potent. A wide variation in toxic activity between strains was reported and it was clear that cell-specific cytotoxic activity was apparent.

4.9 Toxin production has also been examined in a project investigating the virulence potential and invasiveness of *C.jejuni* strains isolated from poultry during a national

survey of broiler flocks (**MAFF, FS3107**). Over 2000 *Campylobacter* strains have been isolated, speciated and partly genotyped. A database of these isolates and their characteristics has been compiled. The strains are currently being screened using several *in vitro* virulence assays. The cytolethal distending toxin genes (*cdt*) are very conserved so an immunoassay is currently being developed to detect variations in expression of CDT. The role of CDT and other toxins (cytotoxin, enterotoxin, Shiga-like toxin and haemolysin) in *Campylobacter* pathogenesis remains unclear and the clinical significance of toxin production is still largely unknown.

4.10 As there is preliminary evidence that lipopolysaccharide (LPS) plays a role in virulence, one funded project has investigated the genetic basis for the production and variation of LPS in *C.jejuni* (**DH, 225**). The aim of the project was to determine the role of LPS in phagocyte killing, cell adhesion/colonisation, and cell invasion using mutants defective in specific stages of LPS synthesis. DNA fragments containing putative LPS synthesis genes were isolated from 2 strains of *C.jejuni*. Up to 7 open reading frames (orfs) were present and designated *orfA* - *orfG*. Mutational analysis has been carried out on 3 of these genes to determine their contribution to the production of LPS. Two of the mutations had no detectable effects on LPS production, while the third altered the reactivity of the core moiety to antiserum indicating that it may be involved in core biosynthesis. Manipulation of *orfE* demonstrated that the organisation of the locus around the gene is important in the production of the O-antigen of repeating sugar units. A studentship project has also investigated the genetic characterisation of *Campylobacter* lipopolysaccharide biosynthesis (**BBSRC, 96/A1/D/02255**). A better understanding of the genetic basis for the production and variation of LPS should provide information on the role of LPS in virulence and, as they are known to confer a degree of resistance to host defences upon bacteria, host specificity.

Adhesion and colonisation

4.11 The virulence, adhesion and colonisation properties of *Campylobacter* strains isolated from humans, chickens and cattle have been investigated (**DH, 228**). The strains were tested in an adhesion and invasion assay and examined with HeLa, Vero and CaCo-2 cell lines, in a chick embryo lethality assay (11-day-old), and in a mouse ligated ileal loop assay. All the isolates demonstrated haemolytic activity but not potent cytolytic activity (discussed further in paragraph 4.8). The adhesion and invasion assay revealed considerable differences among the strains in their ability to adhere and invade the 3 cell lines. The total numbers of *C.jejuni* strains that adhered to the tissue cells did not invade the cells synchronously during the incubation period. The strains were most active against the CaCo-2 cells. Using the chick embryo lethality assay, there was more growth in the embryos inoculated with a low dose and more death of *C.jejuni* in embryos inoculated with a high dose. It would appear that there are different systems in operation for the low and high dose inocula and the researchers have postulated that the high inoculum doses may cause endotoxic death. Using the mouse ileal loop assays, differences between the pathology scores of strains (e.g. fluid accumulation in the loops) were observed. However, when the ileal loop pathology was compared to cytotoxicity and tissue cell adhesion/invasion, there was no statistical correlation between the tests. The researchers concluded that the *in vivo* assays were monitoring different effects and the *in vitro* assays were not a replacement for the *in vivo* assays. It

would therefore appear that phenotypic properties exhibited by the strains after *in vitro* growth may differ markedly from those produced *in vivo*. The widely different phenotypes of individual strains, even from the same source, suggests a wide genetic diversity in virulence-associated loci. The nature and regulation of the virulence-associated properties that promote varying degrees of pathogenicity *in vivo* remain to be elucidated.

4.12 A research project investigating the viable non-culturable (VNC) state in *Campylobacter* has demonstrated that nutrient stress greatly reduces the infectivity of campylobacters (**DH, 132A**). The type of stress and the way the organism has been handled prior to culture influences this reduction in infectivity (see section 3.7 for details of reduction in survival and culturability). The techniques developed were used to produce defined populations of VNC forms from four *C.jejuni* strains. In no case did the VNC forms colonise one-day-old chicks using dose levels ten times greater than that expected to colonise 100% of chicks. The research also indicated that the culture conditions used to produce the VNC forms could have significant effects on the colonisation potential of *C.jejuni*. Two dimensional electrophoresis indicated that these changes may be reflected in the expression of certain novel polypeptides. Given the changes observed in colonisation potential such novel polypeptides may have a role in the virulence of the organism.

Animal model of Campylobacter infection

4.13 Work has been funded to provide an animal model of *Campylobacter* infection relevant to the human disease which, when fully established would enable a variety of experiments on the pathogenic mechanisms of *Campylobacter* to be conducted (**DH, 224/224B**). The work has demonstrated that adult immunodeficient mice become heavily colonised after intragastric inoculation (10^7 - 10^9 cfu g⁻¹), compared to much lower colonisation levels in immunocompetent mice. When inoculated with one of a number of fresh clinical isolates of *Campylobacter*, approximately 10-20% of the animals became ill with diarrhoea and displayed histopathological lesions typical of human campylobacteriosis. Severe pathology was limited to the large intestine and was suggestive of an acute, bacteria-induced inflammation. These signs of disease often took long periods (up to 5 months) to develop. Complementary studies have demonstrated the usefulness of the model in studies of tissue invasion of *Campylobacter* strains, including defined virulence mutant. Using the model, *C.jejuni* CDT was found to have a significant effect on host tissue invasion. The model has also provided information on the potential for, and progression of, multiple infections by *Campylobacter* strains and their apparent capacity to exchange genetic information *in vivo*, particularly antibiotic resistant determinants.

MSFFG Assessment and Identification of Gaps

4.14 There are a lot of gaps in our understanding of the pathogenic mechanisms of *Campylobacter* and a number of virulence factors may be associated with human disease. The research has demonstrated considerable variation in virulence among

Campylobacter strains, the full extent of which remains to be determined. The mechanisms by which campylobacters cause disease are still not established. It is still not clear why campylobacters cause disease in humans and in some animals but not in other animals, but some research is underway to address this. A model of *Campylobacter* infection has been developed and, when fully established, will be an extremely useful resource for understanding the pathogenic mechanisms of *Campylobacter*. In addition, researchers should be encouraged to make best use of the data arising from the *Campylobacter* genome project.

4.15 Additional gaps in funded research were identified as follows:

- Latest research has called into question the validity of *in vitro* assays for monitoring pathogenicity as they appear to monitor different effects to *in vivo* assays. Further research using *in vivo* assays should therefore be encouraged
- The role of toxins in human disease (e.g. cytotoxin, enterotoxin, shiga-like toxin, cytolethal distending toxin and haemolysin) remains unclear and further research may clarify the clinical significance of toxin production
- Are VNC forms a food safety problem? Funded research suggests these forms lack the ability to colonise the gut, but results to the contrary have been published. Further research and use of the animal model for *Campylobacter* may clarify this issue
- Establishment and validation of an animal model which is suitable for studying *Campylobacter* infections.

5. EPIDEMIOLOGY

Introduction

5.1 The ACMSF (1) made several recommendations relevant to the epidemiology of these organisms in animals and humans. These included the need for population studies to assess the real magnitude of campylobacteriosis in humans, the level of human immunity, the origins of infections, and the routes of transmission in animals and humans. The ACMSF recognised that development of subtyping methods was central to gaining a better understanding of the epidemiology including better tracing of sources and transmission routes of human infections. The need for robust subtyping to support our understanding of the epidemiology of *Campylobacter* spp. was reiterated in the ACMSF's report on poultry meat (4).

5.2 The WHO (2) recommended that the relative importance of the different potential sources of human *Campylobacter* infection needs to be elucidated and noted that this may vary from one country to another. The need to establish an expert, international working group, co-ordinated by WHO, to provide advice and support for further epidemiological studies on *Campylobacter* infections in the different countries was recommended. The ultimate aim of the group would be to ensure that there is proper economic justification for national control programmes through cost-benefit analysis.

5.3 In addition to epidemiological analyses of data collected by national surveillance systems, the WHO recommended that statistically controlled studies on the occurrence and cost of human Campylobacteriosis should be carried out in different countries to provide information on how best to allocate funds within the field of public health.

5.4 The Danish Veterinary and Food Administration (3) stated that knowledge on the relative importance of single factors including foods, drinking water, animals and the environment for developing human campylobacteriosis is not optimal. The incidence of *Campylobacter* in environmental reservoirs (e.g. private wells and bathing water) of human significance has currently not been sufficiently examined.

5.5 Fourteen projects fall within the category of epidemiology. These research projects cover the broad areas of animal and human epidemiology. Account has also been taken of the subtyping and surveillance work mentioned in other chapters (see Chapter 2).

Current State of Publicly Funded Research

Campylobacteriosis in humans

5.6 A major study of Infectious Intestinal Disease (IID) in England was conducted between 1993 and 1996 using 70 GP practices in the Medical Research Council's GP Research framework (DH, 177/178). The aim of the study was to estimate the number and aetiology of cases of IID in the population, presenting to GPs and having a stool

sample sent routinely for laboratory examination; to compare these numbers and aetiologies with those recorded by the national laboratory reporting surveillance system; to estimate the prevalence of asymptomatic infection with agents associated with IID; to document differences between cases of IID (in the population and presenting to GPs) and similar but well people (controls) and; to estimate the socio-economic burden of IID and its distribution. *Campylobacter* spp. were isolated from 4.2% of cases in the community and from 12.2% of cases that presented to a GP. Figures for *Salmonella* spp. were 0.4% and 5.0% respectively. *Campylobacter jejuni* and *C. coli* accounted for 88% and 9% of the *Campylobacter* isolates. For every laboratory confirmed case of *Campylobacter* infection reported to national surveillance it was estimated that there were 7.6 cases in the community. This means that if there are 50,000 reported *Campylobacter* infections in England and Wales in a year the actual figure, based on the IID study estimate, would be 380,000.

5.7 Carriage of *Campylobacter* spp. was found in a small percentage of controls in the IID study in England (0.9% in the population) (DH, 177/178). Other work suggests that certain campylobacters may be commensals rather than pathogens. The name *Campylobacter hominis* sp. nov. has been proposed for an undescribed commensal species detected in faecal samples from 10 of 20 healthy individuals (DH, 220B). The organism was initially culture resistant but sequencing and comparison of 16S rRNA *Campylobacter* genus-specific amplicons from faeces suggested a fastidious anaerobe and the organism was eventually cultured after adapting the culture methodology.

5.8 A long-term follow-up study of IID cases is being conducted to ascertain the prevalence of sequelae (e.g. reactive arthritis and irritable bowel syndrome) (DH, 189). This may yield information relevant to *Campylobacter* infection.

5.9 Conventional microbiology (direct plating and/or enrichment) was used to isolate campylobacters in the IID study in England. An alternative approach is being used to look for new or less common campylobacters in human faecal samples (DH, 220). PCR based assays have been developed for 16S rRNA genes sequences for the genus *Campylobacter* and five species (*C.upsaliensis*, *C.helveticus*, *C.fetus*, *C.hyointestinalis* and *C.lari*). PCR assays have shown that it is possible to identify campylobacters in DNA extracted from human faeces (DH, 220B). A PCR-ELISA method was developed which detected and speciated campylobacters directly from faecal DNA extracts including 3,738 faecal samples received by 7 Public Health laboratories from sporadic cases of acute IID in England and Wales over a two year period. While there was no statistically significant difference between PCR (478 samples) and culture (461 samples) in detection of *C.jejuni/C.coli* in these samples, PCR provided unique data about mixed infections and non- *C.jejuni/C.coli* campylobacters. PCR indicated mixed *C.jejuni/C.coli* infections in 19 cases, and *C.jejuni/C.upsaliensis* in 1 case. Eleven cases of gastroenteritis were attributed to *C.upsaliensis* by PCR, 3 cases to *C.hyointestinalis* and 1 case attributed to *C.lari*. This represents the highest incidence of *C.upsaliensis* yet reported from human gastroenteritis, whilst the low incidence of *C.lari* suggests that it is less important in this

context. Current work is focusing on developing greater scope, sensitivity and flexibility based on the LightCycler™ advanced PCR platform (**DH, 220C**).

Prevalence in animals

5.10 There is relatively little UK prevalence data on carriage of *Campylobacter* in food animals although information on faecal carriage in cattle, sheep and pigs will be provided by large prevalence studies being conducted at abattoirs in England, Wales and Scotland during 1999 (**MAFF, FSZ2500 and FT9119**, see paragraph 6.4 for details). Most of the funded research studies relating to *Campylobacter* epidemiology in food animals have focused on poultry, particularly the broiler production chain. Three studies (**MAFF, FS3303; DH, 227; DH, 227B**) have examined/are examining poultry production from the rearing farm up to and including finished poultry meat following processing. Much of the focus has been on using subtyping/fingerprinting as a tool to identify and track changes in the *Campylobacter* populations occurring in these settings. A new project aims to identify critical points for infection of live birds and contamination of poultry carcasses with *Campylobacter* and *Salmonella* (**MAFF, FS3306**). Phenotypic and molecular typing and fingerprinting methods are being used to examine the sources of *Campylobacter* in broiler rearing houses and monitor the spread of infection (**MAFF, FS3306; DH, 227**).

5.11 High resolution molecular genotyping (flagellin gene typing, pulsed field gel electrophoresis) have been used to examine the genetic diversity of campylobacters isolated from human clinical, animal and environmental sources in the North West of England (**DH, 221**). A wide diversity of genotypes were found in the farm environment. Several genotypes were common to cattle, sheep, turkeys and humans whereas others appeared to be host specific.

Origin of infections and routes of transmission

5.12 Risk factors for *Campylobacter* infection in humans have been examined in several studies (**DH, 129; DH, 177/178; DH, 226**). Dogs are known to carry campylobacters and one project (**DH, 129**) has specifically looked at the risk of zoonotic transmission of *Campylobacter* infection from dogs with diarrhoea attending veterinary surgeries. *Campylobacter* spp. were isolated from 32% of faecal samples from dogs with diarrhoea. *C.jejuni* was the most frequent species and prevalence of *Campylobacter* infection was higher in dogs less than 6 months of age (51%) compared to older dogs (7%). An association was found between *Campylobacter* infection in dogs and diarrhoea in human household members, but this was not significant.

5.13 One project has looked at the epidemiology of ciprofloxacin resistance in isolates of *Campylobacter* from human cases of campylobacteriosis in central Southern England (**DH, 226**). Overall 13% of isolates were resistant to ciprofloxacin. Epidemiological analysis indicated that foreign travel (particularly Iberian peninsula), eating or handling uncooked poultry, exposure to untreated water, contact with animals

other than pets and taking medicines before the onset of illness reached statistical significance in a univariate analysis.

5.14 The IID Study in England (**DH, 177/178**) identified travel abroad and eating chicken as significantly significant associations with higher risk of *Campylobacter* disease. Statistically significant associations with lower risk of disease included salad and rice eaten at home, fruit, pulses, pasteurised products and fish. Associations which were not statistically significant, but of interest included a higher risk in those who drank bird pecked milk, ate barbecued chicken, or used antacid, and a lower risk in those who used a domestic water jug.

5.15 A study due to start in November 1999 plans to follow 40 broiler flocks from hatching until packaging and sufficient samples are to be taken to accurately identify sources of infection/contamination. An audit of the impact on immersion scalding on chicken muscle contamination is also planned (**MAFF, FS3306**).

Level of human immunity

5.16 Work has been undertaken to explore the feasibility of developing an immunological assay, which could be used to determine the prevalence of potentially immunologically protected or susceptible individuals (**DH, 186**). Work has identified specific antigens (flagellin, major outer membrane protein (MOMP) and a 40kDa antigen) which elicit a long-term response and are less frequent in control populations. Results indicate that a test could be based on the specific antigens and measuring serum or salivary IgG antibodies as an indicator of long-term immunity to *C.jejuni*.

MSFFG Assessment and Identification of Gaps

5.17 Although a lot of good epidemiological studies have been funded on both the human and animal side but we still do not know to what extent campylobacteriosis is foodborne. There is a need for a unified approach to *Campylobacter* subtyping to support our understanding of the epidemiology of *Campylobacter* spp.. Gaps in our knowledge were identified as follows:

- The vast majority of cases of *Campylobacter* infection in humans are apparently sporadic and outbreaks are rarely identified. What are the reasons for this?
- Why does *Campylobacter* infection occur more frequently in certain populations and not others?
- Why does campylobacteriosis in humans peak in May in the UK?
- What is the extent of immunity to *Campylobacter* infection in humans in the UK?
- What is the source of infection in poultry?

6. SURVEILLANCE

Introduction

6.1 The ACMSF (1) recommended that the Government funded population studies to assess the real magnitude of campylobacteriosis, and further studies of transmission to understand better its seasonality.

6.2 The WHO (2) recommended that national surveillance systems for foodborne disease should include *Campylobacter* infections.

6.3 Four projects fall within the category of surveillance and the research covers the area of food surveillance. Other research covering the prevalence of *Campylobacter* in humans and animals is considered in chapter 5.

Findings of Publicly Funded Surveillance

6.4 A 12-month survey is underway to measure the prevalence of excretion of foodborne pathogens by cattle and sheep presented to abattoirs in Great Britain for human consumption (**MAFF, FSZ2500**). Of the 393 eligible abattoirs which kill cattle and/or sheep, 117 are participating in the study. Twenty percent of the samples of rectal contents are examined for *Campylobacter*, *Salmonella*, commensal *E.coli*, *Yersinia* and *Enterococcus faecium*, and all samples are examined for VTEC O157. A second abattoir survey is underway to measure the prevalence of foodborne zoonotic organisms in pigs slaughtered for human consumption (**MAFF, FT9119**). Thirty-four abattoirs slaughtering pigs are participating in the study with about 2500 samples being collected over a 12-month period. Three types of samples are collected at each visit: caecal contents, carcass swabs and neck muscle samples. All samples are examined for *Campylobacter*, *Salmonella*, VTEC O157, commensal *E.coli*, *Yersinia* and *Enterococcus faecium*.

6.5 A three month pilot study, which finished in March 1999, examined fresh and frozen UK and imported poultry on retail sale (**DH, 271**). Of 101 chickens examined, 94% were *Campylobacter* spp. positive. Isolation rates of *Campylobacter* spp. for neck-skin, chicken surface rinse and chicken surface rinse plus skin samples were approximately 88%, 90% and 93% respectively. The mean *Campylobacter* spp. positive counts were 2×10^3 per g for neck-skin, 2×10^5 per g for chicken surface rinse and 3×10^5 per g for chicken surface rinse plus skin.

6.6 A two year study to determine the prevalence and distribution of *Campylobacter* infecting chicken flocks in selected locations of South West England was completed in 1997 (**DH, 227**). During rearing, broilers were colonised by a number of different *Campylobacter* subtypes and by more than one species although *C.jejuni* predominated. The distribution of subtypes altered during the 42-day growth period with new subtypes being brought into the house probably from the environment. Where other rearing houses that are colonised exist on the same site, their predominant strains may

be transferred to other houses, altering the hierarchy of subtypes that exist within a house. Transportation of flocks to the plant may result in further contamination of the birds by new serotypes via contaminated crates. During processing the predominant subtype on a flock can alter, possibly due to some subtypes being less able to tolerate the heat and other stresses that may be encountered along the production line.

MSFFG Assessment and Identification of Gaps

6.7 The identification of gaps and overlaps in this area is a matter for the Microbiological Food Surveillance Group, the Epidemiology of Foodborne Infections Group and the Veterinary Surveillance Group set up under the CVO. Projects on surveillance will continue to be listed under the MSFFG system, but the work will not be discussed by the Funders Group.

7. RISK ANALYSIS

Introduction

7.1 The WHO (2) noted that risk factors for *Campylobacter* enteritis at different stages of the food-food chain may differ from one country or region to another. WHO recommended that these factors need to be identified and evaluated in each case before effective control of human infection becomes possible. In relation to poultry, it was recommended that risk factors for *Campylobacter* colonisation should be clarified and ranked according to significance. Such risk assessment will help to direct further research efforts.

7.2 The Danish Veterinary and Food Administration (3) recommended the implementation of a risk assessment for *C. jejuni* with the reservation that insufficient data concerning other possible sources of infection, other risk factors, typing methods, etc. may be generated simultaneously with the actual risk assessment. The fact that these data will be forthcoming supports the need for such research otherwise there would not be the necessary scientific basis for the risk assessment itself.

7.3 This section addresses a diverse topic encompassing the three main elements of risk analysis: risk assessment, risk management and risk communication. Projects in this area can potentially be very diverse ranging from risk assessments for *Campylobacter* spp. in a specific food, application of risk management tools such as Hazard Analysis and Critical Control Point (HACCP) and communicating risk to the general public.

Current State of Publicly Funded Research

7.4 A study has been carried out to review information on *Campylobacter* contamination of poultry meat with a view to developing a quantitative risk assessment model (**MAFF, FS3305**). Work has also been undertaken to gather data on numbers and subtypes of campylobacters on retail chilled and frozen chicken to inform exposure assessment for these pathogens (**DH, 271/271B**). An assessment of the risks to human health from the presence of campylobacters in on-farm poultry is being conducted as part of a MAFF studentship.

7.5 A recently started project is attempting to identify the critical points for infection of live birds or contamination of poultry carcasses by *Campylobacter* (**MAFF, FS3306**). Up to 40 flocks will be followed from hatching until packaging of poultry meat.

MSFFG Assessment and Identification of Gaps

7.6 Quantitative risk assessment for *Campylobacter* in the food chain is hampered by the lack of definitive information on the pathways of infection and associated subtypes. However, despite these limitations, risk assessment can be used to identify

factors influencing the exposure of humans to campylobacters and where interventions to reduce this exposure might best be targeted. Gaps were identified as follows:

- The development of a preliminary risk assessment for the exposure of humans to campylobacters in food
- What is the contribution made by cross contamination in the kitchen to human exposure to campylobacters?

8. REDUCTION AND ELIMINATION

Introduction

8.1 The ACMSF (1), made a number of recommendations for Government funded research in the area of reduction and elimination of *Campylobacter* in the food chain. It was recommended that research be undertaken:

- on the prevalence of *Campylobacter* infection in UK poultry flocks, the origins of infection and the routes of transmission, and the mechanisms by which infection may be controlled.
- (by the industry) to examine the effect of irradiation on *Campylobacter* species (other than *C.jejuni*) associated with human disease.
- (by the industry) to investigate the effect of preservatives and the use of modified atmospheres in processing and packaging on the survival or inactivation of campylobacters in food.

8.2 The first of these recommendations was highlighted again by the ACMSF in its 1996 Report on Poultry Meat (4).

8.3 The WHO (2) recommended the development of an effective but relatively inexpensive vaccine against *Campylobacter* to supplement conventional hygiene precautions on the farm. As an alternative to the vaccine approach, or even an additional treatment, the WHO recommended the introduction of an antagonistic microflora into the alimentary tract. Research to isolate, identify and test potentially antagonistic bacteria needs to be carried out, taking account of possible effects on bird growth performance.

8.4 The WHO noted that epidemiological studies appear to suggest that repeated exposure to campylobacters induces protection against illness but not colonisation. It was recommended that the immunological implications of this phenomenon should be studied further with a view to developing a vaccine for use in underdeveloped countries, where human campylobacteriosis is a significant cause of mortality.

8.5 Six projects currently fall within the category of reduction and elimination. These can primarily be split between those projects aimed at 'primary production' (i.e. on-farm) and those at the slaughterhouse. All the projects have the common aim of seeking means of reducing the proportion of *Campylobacter* positive poultry on retail sale in the UK.

Current State of Publicly Funded Research

8.6 A project that was completed in 1998 (**MAFF, OZ0130**) sought to investigate the possibility of genetically engineering a strain of *C.jejuni* to remove its virulence factors. This strain would then be used to prevent birds becoming colonised with wild-type strains. This work on competitive exclusion has continued with the same contractor

(**MAFF, OZ0603**), with further work using an experimental oral chick model being undertaken to investigate how well competitive exclusion works.

8.7 A study aimed at developing strategies to reduce or eliminate *C.jejuni* colonisation in chickens (**MAFF, OZ0129**) has investigated potential vaccine candidate antigens. It was concluded that the most effective option for a vaccine would be based on a live *Campylobacter* strain. A number of mutant strains were investigated in chicken colonisation studies. Further work is needed before an effective vaccine can be produced and this line of research continues in a current project (**MAFF, OZ0603**).

8.8 An in-depth study of the molecular epidemiology of *Campylobacter* in broiler houses is being carried out (**MAFF, FS3303**). This has already shown that unauthorised staff entry routes are a primary cause of infection and improved hygiene procedures have been suggested with some initial success. Birds do appear to be inherently resistant to challenge with *Campylobacter* during the lag time in the broiler house, with maternal immunity perhaps being more important than the gut flora.

8.9 A project tracing *Campylobacter* through poultry processing has been completed (**MAFF, FS1052**). This found that it was often the case that there were multiple *Campylobacter* strains found within the slaughterhouse, not all of which could be traced back to incoming flocks. The study highlighted the importance of being aware that the predominating faecal strain or strains could mask the presence of other strains present in very low numbers. Sources of cross-contamination found were the stun water, not washing or changing gloves between flocks, and the packing area surface where a deposit of slime can build up.

8.10 The detachment of *Campylobacter* from poultry carcasses has been investigated (**MAFF, FS1314**). The work, which has been completed, aimed to reduce contamination levels by preventing attachment of the organism. It was shown that the adherence of *Campylobacter* spp. to poultry skin appeared to be mediated by physical means, with the possibility of entrapment in the structure of the skin, and the production of structures by the bacteria which promote adhesion. Therefore it appears that disruption/degradation of the skin would be required in order to improve cell release.

MSFFG Assessment and Identification of Gaps

8.11 At present there is no reliable consistent means of reducing *Campylobacter* infection in primary animal production and there is a lack of a reduction strategy in the slaughter-house and beyond. The following gaps in our knowledge have been identified:

- What is the source of *Campylobacter* infection in poultry houses?
- How can *Campylobacter* infection be reduced at the farm level?
- What role can vaccines and other intervention steps (e.g. phage exclusion work) play in a reduction strategy?

9. MICROBIAL ANTIBIOTIC RESISTANCE

Introduction

9.1 The ACMSF has considered the complex issue of microbial antibiotic resistance in relation to food safety and made a wide range of recommendations for research in its recently published report (5). The ACMSF regarded two areas as particularly important:

- Work is needed on the chain of events which can lead to antibiotic-resistant microorganisms arising from farming practices, being transmitted through food chain pathways, and causing human infection
- Research is very much needed on possible exposure of general, animal and food microbial flora to resistance, with the accompanying risk of the establishment of a reservoir for the transfer of such resistance to humans

9.2 To date, only one project falls exclusively within this category although antibiotic testing forms a part of 2 other projects. It is possible that the number of projects in this category will increase in the future as funders commission work to address recommendations following publication of the ACMSF Report.

Current State of Publicly Funded Research

9.3 A local study in central southern England investigated the prevalence of ciprofloxacin resistance in campylobacters isolated from humans, retail fresh chicken and chickens at slaughter (**DH, 226**). Overall, 13% (153/1219) of campylobacters from humans and 11% (9/82) of campylobacters from retail fresh chicken were classed as resistant to ciprofloxacin (MIC>32 mg L⁻¹). Epidemiological analysis revealed that ciprofloxacin-resistant *Campylobacter* infection in humans was significantly associated with foreign travel, eating or handling uncooked poultry, exposure to untreated water, contact with animals other than pets and taking medicines before the onset of illness.

9.4 Antibiotic profiles will be determined for selected *Campylobacter* isolates from a current study measuring the prevalence of foodborne zoonotic organisms in pigs at slaughter (**MAFF, FT9119**). Information on antibiotic resistance in *Campylobacter* from poultry meat will also be gained from a pilot survey of *Salmonella* and *Campylobacter* spp. in retail poultry (**DH, 271B**).

9.5 This year will see the start of several projects on antimicrobial resistance that will include studies of *Campylobacter*. Further research in this area may be funded in response to the recommendations of the ACMSF report on microbial antibiotic resistance (5). This area will be assessed at a future date.

10. OTHER

Introduction

10.1 The WHO (2) recommended that, in addition to epidemiological analyses of data collected by national surveillance systems, statistically controlled studies on the occurrence and cost of human campylobacteriosis should be carried out in different countries to provide information on how best to allocate funds within the field of public health.

10.2 To date, one project has been placed in this category.

Current State of Publicly Funded Research

10.3 A major study of infectious intestinal disease (IID) in England was conducted between 1993 and 1996 using 70 GP practices in the Medical Research Council's GP Research framework (DH, 177/178). One of the aims of the study was to estimate the socio-economic burden of IID and its distribution. It was estimated that the total cost of cases of IID in England during the study period was £742.8 million or £78.89 per case. The NHS costs were 37% of these costs. Using an alternative assumption for the costs based on the estimated cost of those who did not see a GP in the community component and those who saw a GP in the GP component study, the cost was £676.9 million. The cost estimated on this basis was £69.5 million for *Campylobacter*. This compares to £69.3 million for enterovirulent *E.coli*, £46.4m for *Salmonella*, 24.4m for SRSV, £16.5m for rotavirus and £5.6m for *C.difficile*.

MSFFG Assessment and Identification of Gaps

10.4 Projects falling into this area of research, e.g. the socio-economic costs of foodpoisoning outbreaks and sporadic cases, provide information on the impact of food poisoning on society. The following gaps in our knowledge have been identified:

- What are the socio-economic costs of sequelae associated with *Campylobacter* infection, in particular Guillain-Barré syndrome

REFERENCES

1. Advisory Committee on the Microbiological Safety of Food. Interim Report on *Campylobacter*. (1993); HMSO, London
2. The World Health Organization. Report of WHO consultation on epidemiology and control of campylobacteriosis in animals and humans. (1994); Food Safety Unit, World Health Organization, Geneva, Switzerland
3. The Danish Veterinary and Food Administration. Risk profile for pathogenic species for *Campylobacter* in Denmark. (1998); The Division of Microbiological Safety, The Danish Veterinary and Food Administration, Denmark
4. Advisory Committee on the Microbiological Safety of Food. Report on poultry meat. (1996); HMSO, London
5. Advisory Committee on the Microbiological Safety of Food. Report on microbial antibiotic resistance in relation to food safety. (1999); The Stationery Office, London

GLOSSARY

Autochthonous

Refers to any population which is indigenous to a given environment. Autochthonous microbes generally maintain more or less constant biomass and numbers.

CaCo-2 cells

Human colon adenocarcinoma cells. This is a human intestinal epithelial cell line which differentiates after forming a confluent monolayer to produce a single, polarised layer of cells with typical brush border microvilli on the apical surface.

Campylobacter

A curved Gram-negative, non-sporing bacterium. There are two principal species that cause human disease, *C. jejuni* and *C. coli*.

Cholera-Like Toxin

A toxin which acts in the same way as cholera toxin to produce symptoms of profuse, watery diarrhoea. Cholera toxin comprises two subunits designated A and B. Subunits of B bind to the brush border of cells lining the intestinal tract and form a channel through which subunit A passes.

Citric acid cycle

A cyclic sequence of reactions that plays a central role in the metabolism of many microorganisms and involves several tri-carboxylic acid intermediates.

Culturable/non-culturable

Refers to an organism which can/cannot currently be grown in a culture medium.

D-value

The time required at a given temperature to reduce the number of viable cells or spores to 10% of the initial number.

Flagellin

The protein subunit of the filament of a bacterial flagellum.

Guillain-Barré syndrome

A disorder characterised by acute onset of weakness in the distal muscles of the legs which spreads upward over the course of a few days to involve the trunk, arms and sometimes the cranial nerves. The syndrome is an uncommon complication of *Campylobacter* infection.

HeLa cells

A cell line derived from a human cervical carcinoma.

Irritable bowel syndrome

A condition in which unusual motility of both the small and large bowels produce discomfort and intermittent pain, with no known cause.

Lightcycler™

An advanced PCR platform that allows quantitative detection of PCR products.

Lipooligosaccharide (LOS)

A shorter version of the LPS component of Gram negative organisms, comprising the lipid A anchor and central core but lacking the O-antigen. *Campylobacter* is known to produce both the full-length LPS and the shorter LOS.

Lipopolysaccharide (LPS)

The endotoxic component of the outer membrane in Gram negative organisms and is antigenically variable. LPS is composed of a lipid A anchor, a core of non-repeating sugar subunits and an O-antigen of repeating sugar subunits.

Methyl-accepting chemotaxis proteins

Proteins which enable a bacterium to respond to changes in the concentration of a chemical (chemoeffector), via transduction of a signal across the cytoplasmic membrane.

Microaerophilic

A gaseous environment in which oxygen is present at a concentration (partial pressure) significantly lower than in air.

Mitochondria

A semi-autonomous intracellular organelle present in eukaryotes. Respiration and the reactions of the citric acid cycle occur within mitochondria.

PFGE

Pulsed-Field Gel Electrophoresis. This technique separates DNA molecules by subjecting them to alternately pulsed, perpendicularly placed electrical fields.

Phagocyte

Any of a class of cells, particularly neutrophils and macrophages, which are able to ingest particulate matter. This may then be digested by the cell.

Phospholipase A

Phospholipases are a class of enzymes that hydrolyse phospholipids resulting in the release of fatty acids.

RAPD

Randomly Amplified Polymorphic DNA. This technique uses much shorter primers than conventional PCR and the primer sequences are chosen at random, thus no prior knowledge of the genome sequence is required. Variation in RAPD profiles arise from the insertion or deletion of DNA.

RFLP

Restriction Fragment Length Polymorphism. A technique used to distinguish between subtypes of bacteria on the basis of differences in DNA sequences and thus the size and number of restriction fragments generated.

RT-PCR

Reverse-Transcriptase Polymerase Chain Reaction. This is a variation of the standard PCR technique which uses the enzyme Reverse Transcriptase to produce a DNA molecule from an RNA template.

Socio-economic burden

The financial and other costs, including effects on quality of life, as a result of disease.

Southern hybridisation

A procedure used to detect specific sequences in DNA that has been cleaved into fragments by restriction endonucleases. The fragments are initially separated by electrophoresis then denatured, blotted onto a medium and then exposed to a sequence-specific probe.

Superoxide dismutase

Any of a range of metalloenzymes that catalyse the formation of superoxide into peroxide and oxygen. The enzyme protects aerobic organisms from the toxic effects of superoxide.

Thermophilic group

Refers to those campylobacters which grow well at 42°C and 37°C but not at 25°C.

Vero cells

A cell line derived from African Green Monkey Kidney cells and gives its name to the cytotoxins produced by some Enterovirulent strains of *Escherichia coli*.

Viable

Refers in microbiology to an organism capable of reproducing under appropriate conditions.

APPENDIX 1.2

A 1.2 Research and Development projects considered in the discussion paper

Project Code	Project Title	Start Date	End Date	Funder
BFP11346	Respiration and oxygen tolerance in <i>Campylobacter coli</i> and <i>C. jejuni</i> : Implications for food safety and colonisation	07/01/99	07/01/02	BBSR C/SER AD
BFP11362	Construction and application of a <i>Campylobacter jejuni</i> DNA microarray to investigate differential gene expression	07/01/99	07/01/02	BBSR C/SER AD
BFP11294	An analysis of carbon metabolism and alternative respiratory pathways in <i>C.jejuni</i>	10/01/99	10/01/02	BBSR C/SER AD
BFP11390 (joint with BFP11391 & BFP11392)	Post-genomic analysis of the proteome of <i>Campylobacter jejuni</i>	07/01/99	07/01/02	BBSR C/SER AD
BFP11391(j oint with BFP11390 & BFP11392)	Post-genomic analysis of the proteome of <i>Campylobacter jejuni</i>	07/01/99	01/01/02	BBSR C/SER AD
BFP11392 (joint with BFP11390 and BFP11391)	Post-genomic analysis of the proteome of <i>Campylobacter jejuni</i>	05/01/99	05/01/02	BBSR C/SER AD
D09207	The <i>Campylobacter jejuni</i> RacR regulatory system: Characterising the regulon and its role in adaptive responses and intestinal colonisation	02/28/98	02/28/01	BBSR C
3250762	The use of host specificity to identify virulence determinants in <i>Campylobacter</i> spp	02/05/96	02/04/99	BBSR C
3258371	Microbial adaptation to environment and survival in the food chain	04/01/98	03/31/01	BBSR C
96/A1/D/022 55	Genetic characterisation of <i>Campylobacter</i> lipopolysaccharide biosynthesis (A Studentship Project)	09/23/96	09/22/99	BBSR C
98/A1/P/042 61	Characterisation of the iron regulon of <i>Campylobacter jejuni</i> (A Studentship Project)	09/28/98	09/27/01	BBSR C
96/B1/D/024 08	Cold adaptation and culturability in <i>Campylobacter jejuni</i> (A Studentship Project)	09/30/96	09/29/99	BBSR C

98/B2/D/04041	Outer membrane proteins of <i>Campylobacter jejuni</i> : Characterisation and biological role (A Studentship Project)	09/28/98	09/27/01	BBSRC
DANI9641	Molecular typing of <i>Campylobacter</i> species.	01/01/97	01/01/00	DANI
DANI9723	Microbial quality of beef carcasses in Northern Ireland abattoirs - A baseline study.	01/01/97	01/01/99	DANI
112	Analysis of the control of expression and mechanism of action of <i>Campylobacter</i> toxins in relation to virulence and antitoxin probe design	04/01/92	03/31/95	DH
132A	The role of non-culturable forms in the survival and pathogenicity of campylobacters in foods	12/01/92	11/30/94	DH
132B	Characterisation of the environmental factors involved in the culturability and viability of <i>Campylobacter jejuni</i>	04/01/93	03/31/95	DH
159	Development of <i>Campylobacter</i> subtyping	11/02/93	02/01/96	DH
159A	Development of <i>Campylobacter</i> subtyping - Collaboration project	12/01/93	02/01/96	DH
159C	Development of <i>Campylobacter</i> subtyping - Collaboration project	01/01/94	02/01/96	DH
168	Survival, growth and adaptation of <i>Campylobacter jejuni</i> in the environment	04/01/94	05/31/96	DH
176	An investigation into methods for the isolation of campylobacters from the environment	02/01/93	04/30/93	DH
186	Non-invasive measures of <i>Campylobacter</i> immunity : Development, validation and utilisation	01/01/95	12/31/97	DH
220	Molecular genotypic identification of campylobacters and application to clinical material for surveillance	02/01/95	01/31/97	DH
220B	The prevalence of <i>Campylobacter</i> in human disease- A molecular approach	03/01/97	02/28/99	DH
220C	" Real time" PCR detection and speciation of <i>Campylobacter</i>	04/01/99	03/31/00	DH
221	The use of DNA technology to trace the spread of campylobacters from the farm environment	01/01/95	12/31/97	DH
222/222B	Quantitative detection of metabolic <i>Campylobacter</i> spp. in 4 hours (phage amplification)	01/01/95	12/31/97	DH
223	Characterisation and distribution of virulence genes in <i>Campylobacter</i> spp.	02/01/95	01/31/97	DH
224/224B	Development of an animal model of <i>Campylobacter</i> infection relevant to the human disease	01/27/95	06/30/98	DH

225	Genetic analysis of <i>C. jejuni</i> LPS biosynthesis and investigation of the role of LPS in virulence	01/01/95	12/31/98	DH
226	Estimation of the prevalence of quinolone resistance in gastrointestinal pathogens	03/01/95	02/28/96	DH
227	Investigation of the prevalence and distribution of <i>Campylobacter</i> subtypes in poultry	01/01/95	02/28/97	DH
228	An analysis of the spectrum of virulence of <i>C. jejuni</i>	01/01/95	12/31/96	DH
271	Surveillance of <i>Salmonella</i> spp. and <i>Campylobacter</i> spp. in retail UK and imported poultry on retail sale - pilot study	10/12/98	01/11/99	DH
FS1052	<i>Campylobacter</i> spp traced through poultry processing using conventional, molecular and a conductance typing technique	12/01/96	12/01/97	MAFF
FS1214	Development of a rapid PCR approach to the Screening of foodstuffs for the presence of <i>Campylobacter jejuni</i>	01/01/94	12/31/95	MAFF
FS1217	Development of a novel identification and typing system for campylobacters based on repeat sequences with other typing schemes	04/01/93	03/31/96	MAFF
FS1242	Rapid detection, quantification and thermophilic campylobacters in foodstuffs and related environments	09/01/96	08/31/99	MAFF
FS1254	Validation of improved detection methods for the detection of thermotolerant <i>Campylobacter</i>	04/01/97	03/31/99	MAFF
FS1314	Detachment of <i>Campylobacter</i> from poultry carcass surfaces	04/01/94	03/31/96	MAFF
FS1509	Molecular basis of oxygen sensitivity of <i>Campylobacter</i> spp.	04/01/93	03/31/96	MAFF
FS1525	Recovery of full viability in dormant cells of <i>Campylobacter</i>	02/01/95	03/31/98	MAFF
FS1526	Investigation of the physiological mechanisms for the adaptation of campylobacters to stress injury	04/01/95	03/31/98	MAFF
FS3107	Variations in the virulence of <i>Campylobacter jejuni</i> strains associated with poultry and poultry meat	04/01/98	03/31/01	MAFF
FS3303	The molecular epidemiology of campylobacters in poultry and poultry meat and use to develop intervention strategies	04/01/97	03/31/00	MAFF
FS3305	A review of measures to reduce levels of <i>Salmonella</i> and <i>Campylobacter</i> in poultry and Development of an appropriate risk assessment Model	09/01/98	08/31/99	MAFF
FS3306	Studies to identify critical points for infection of live birds or contamination of poultry carcasses with <i>Campylobacter</i> and	11/01/99	06/03/02	MAFF

	<i>Salmonella</i>			
FSZ2500	Survey to measure the prevalence of excretion of foodborne pathogens by cattle and sheep presented to abattoirs in Great Britain for slaughter for human consumption	01/01/99	31/07/00	MAFF
FT9119	Survey to measure the prevalence of foodborne zoonotic organisms in pigs at slaughter	03/01/99	12/31/99	MAFF
OZ0129	The Development of Vaccines Against <i>Campylobacter jejuni</i> in Chickens.	04/01/95	03/31/98	MAFF
OZ0130	Competitive Exclusion of <i>Campylobacter jejuni</i> in Chickens.	04/01/95	03/31/98	MAFF
OZ0140	Veterinary <i>Campylobacter</i> Reference Facility	01/01/98	03/31/98	MAFF
OZ0601	To investigate the non-typability of campylobacters using the LEP Scheme	12/01/98	03/31/99	MAFF
OZ0602	To identify and characterise campylobacters virulent for humans and derived from food producing animals	04/01/98	03/31/01	MAFF
OZ0603	The development of non-pathogenic strains of <i>Campylobacter jejuni</i> as agents of competitive exclusion in poultry	04/01/98	03/31/01	MAFF
OZ0604	Characterisation of strain variation in <i>Campylobacter jejuni</i>	04/01/99	03/31/02	MAFF
OZ0605	Genotypic and phenotypic instability of campylobacters from environmental, animal and human sources	08/01/99	07/31/02	MAFF
URG/001/96	Solid phase rapid detection of viable micro-organisms using nucleic acid amplification and biosensing techniques	04/01/96	09/30/99	SERA D

Total Number of projects = 60

APPENDIX 1.3

A 1.3 Research and Development projects which started after the completion of the discussion paper

Project Code	Project Title	Start Date	End Date	Funder
271B	Methods used for the assessment of the number and prevalence of <i>Salmonella</i> spp. and <i>Campylobacter</i> spp. on retail chickens	11/1/99	1/31/2000	DH
294	Drug resistant screening of <i>Campylobacter</i> isolates from the IID Study	01/01/00	31/03/00	DH
FS3503	Pathogens in organic wastes: their levels and survival both during storage & following application to agricultural land	01/07/99	31/12/02	MAFF
FS3506	The levels of pathogens in abattoir wastes	01/11/99	30/09/01	MAFF
FSZ2500	Survey to measure the prevalence of excretion of foodborne pathogens by cattle and sheep presented to abattoirs in GB for slaughter for human consumption	1999	2000	MAFF
FT9119	Survey to measure the prevalence of foodborne zoonotic organisms in pigs at slaughter	1999	2000	MAFF

Total Number of Projects = 6

Last updated: 12 April 2000

A 1.4 Food Surveillance Projects.

Project Title	Year	Funder
Campylobacter in poultry	1997	DANI
Microbiological analyses of beef cattle in six of the nine EU-approved abattoirs in Northern Ireland - Phase II	1997-1998	DANI
Microbiological analysis of raw goats' milk	1998	DANI
Microbiological quality of cooked ham	1994	DANI (DOE; DCs)
Surveillance of the Microbiological Status of Raw Cow's Milk on Retail Sale	1995-1996	DH
Campylobacter in Food and Water	1993-1994	DHSS/NIPHL
Salmonella and Campylobacter in raw chickens	1996-1998	NIPHL
Campylobacter in Foods and Waters	1993-1994	NIPHL/DCs
Salmonella and Campylobacter in raw poultry	1995-1996	NIPHL/DCs
Salmonella and Campylobacter in raw shellfish	1994	NIPHL/DOE (NI)/DCs
Study of mechanically recovered meat	1995-1996	MAFF
RTE National Study: Part 2	1996	MAFF
RTE National Study: Part 3	1996	MAFF
RTE National Study: Part 4	1996	MAFF
Study of unpasteurised sheep and goats' drinking milk	1997-1998	MAFF
Study of unpasteurised sheep, goats' and buffaloes' milk	1998	MAFF

Total number of projects = 16

Last updated: January 2000

A 1.5 Organisations which constitute the membership of the Microbiological Safety of Food Funders group (MSFFG)

BBSRC	Biotechnology & Biological Science Research Council
DANI	Department of Agriculture for Northern Ireland
DH	Department of Health
DHSS, NI	Department of Health and Social Services, Northern Ireland
FSA	Food Standards Agency (from 1 April 2000)
MAFF	Ministry of Agriculture, Fisheries and Food
SERAD	Scottish Executive Rural Affairs Department
SODoH	Scottish Office Department of Health