

**UK Publicly Funded Research Relating to
Campylobacter: Update, 2004**

Research covered from 1999 to 2003

**Report to the Microbiological Safety of Food
Funders Group**

May 2004

UK Publicly Funded Research Relating to *Campylobacter*: Update, May 2004

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SUMMARY

The report *UK publicly funded research relating to Campylobacter* has been updated for the microbiological safety of food funders group (MSFFG). The new report covers research funded by the members of the MSFFG in a total of 89 projects. These include a range of research areas, the most significant being the development of assays for the genomic classification of different strains and types of *Campylobacter*, research into the genetics and physiology of *Campylobacter jejuni*, studies on the pathogenicity and epidemiology of the organism and considerations of the risks associated with campylobacters and how to reduce these.

Significant progress has been made in the development of the necessary methodologies for, and construction of, classification schemes which are related to the genotype of the *Campylobacter* as well as, or in place of, the phenotype. Ensuring that there is agreement between researchers and other interested parties as to the most appropriate assays and classification schemes is now essential. The evidence of this report is that the current core classification system for *Campylobacter* species should be the multi locus sequence typing (MLST) scheme.

Major progress has also been made in the basic understanding of the genetics and physiology of *C. jejuni*, driven by the availability of the genome sequence and the development of various resources derived from it. The success of this work is considerable and will develop further, in particular if the resources now being developed are used by a wide variety of researchers.

Less progress has been achieved in the areas of pathogenicity, epidemiology and immunology. This may partly reflect the need for clear classification systems and a greater understanding of the function of individual genes. The wide variety of studies required in order to answer questions relating to epidemiology may also have restricted progress in this area.

Campylobacter spp.. remain highly important zoonotic pathogens infecting approximately 1% of the UK population each year. There are many vehicles of infection, which include unpasteurised milk and untreated water. One important route is poultry meat, principally chicken. It has been found that around half of the chicken carcasses introduced into the UK food chain may be contaminated with *Campylobacter*, with the possible risk of the pathogen being introduced to man. This is of relevance to the food standard agency's consideration of reduction and control of *Campylobacter* infections in man.

LAY SUMMARY

Campylobacter is a bacterium which is the most common cause of food poisoning in the UK and worldwide. It has been found mainly in poultry, red meat, unpasteurised milk and untreated water. Although it does not grow in food, it spreads easily, so that only a few bacteria in a piece of undercooked chicken could cause illness. *Campylobacter* infections do not usually cause vomiting, but diarrhoea can be severe and bloody, with abdominal cramps. Normally the treatment is rest and fluid replacement, with medication only being given for severe cases.

This report describes the research which the UK government has funded in the last four years in order to increase our understanding of *Campylobacter* and the illness it causes. In particular, the research covers work to identify the different types of *Campylobacter* which are found in people, chickens, other animals and the environment (because there are many different but very similar types) and research to understand more about the organism itself. Much of this work has benefited from having the *Campylobacter* genome sequence (DNA instruction manual needed by the organism in order to live, grow and replace itself).

The report also includes a significant body of research which seeks to discover how *Campylobacter* infections occur in people. The broad conclusion at this stage is that an important source of these infections is the consumption of contaminated and undercooked poultry meat.

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INTRODUCTION

1. REASONS FOR THE REPORT

- 1.1 In January 2000 the Microbiological Safety of Food Funders Group (MSFFG) published a report entitled: *UK Publicly-Funded Research Relating to Campylobacter* (the 1999 MSFFG *Campylobacter* report). This report provided an overview of *Campylobacter* related research being undertaken in the UK and funded by the members of the MSFFG. The report summarised publicly funded research relating to *Campylobacter* and the microbiological safety of food. The progress achieved in *Campylobacter* research in the UK addressed under a series of research topics was then considered. Finally, the 1999 MSFFG *Campylobacter* report then summarised those aspects of each research area where further work might be needed.
- 1.2 Four years later, it is appropriate for the 1999 MSFFG *Campylobacter* report to be updated. There are particular reasons for this. In 2000 the complete genome sequence of *Campylobacter jejuni* was published (Parkhill *et al* (2000)¹), the sequence itself having been available on the web since late 1999 (www.sanger.ac.uk). It is valuable to see how the introduction of this significant resource is influencing *Campylobacter* research. Following from this, there has been substantial investment in genomic resources for *Campylobacter* through members of the MSFFG and it is again important to see the use to which this investment is being made. Finally, there is currently considerable interest in *Campylobacter* research through the *Campylobacter* Working Group of the Advisory Committee on the Microbiological Safety of Food (ACMSF) and within the FSA, with respect to the development of a strategy for the control of *Campylobacter* in chickens (The Strategy for the Reduction of *Campylobacter* in Broiler Flocks (2003)). A draft/interim report on *Campylobacter* was presented to the ACMSF by the *Campylobacter* Working Group in 2002 (ACMSF Annual Report, (2002)). The ACMSF *Campylobacter* Working Group is expected to present the final version of its report to the Committee in early 2004. This report by the MSFFG may complement the findings of the Working Group. In addition, whilst *Campylobacter* is the most commonly reported cause of food poisoning in the UK (Incidence and Trends in Foodborne Diseases in the UK in the years 2001-2002) and world-wide (Blaser, (1997)), the level of knowledge and understanding of the micro-organism is still limited, and contains significant gaps.
- 1.3 This report provides an overview of progress in publicly funded *Campylobacter* research in the UK. The principal research areas are: the genomic classification of different strains and types of *Campylobacter* (section 4 of the report); research into the genetics and physiology of *Campylobacter jejuni* (section 5); studies on the

¹ Funded by the Wellcome Trust under the Beowulf Genomics programme

pathogenicity (section 6) and epidemiology (section 7) of the organism; and considerations of the risks associated with campylobacters and how to reduce these risks (sections 8–10). For each research area, the report also summarises the gaps in research identified previously, in the MSFFG 1999 *Campylobacter* report. In addition, it identifies those areas where either there is overlap in the research supported or where there are currently significant gaps in knowledge and activity.

2. METHODOLOGY

- 2.1 This report is based, as was the 1999 MSFFG *Campylobacter* report, on those projects which are funded by the members of the MSFFG. These are the Food Standards Agency (FSA), the Department for Environment, Food and Rural Affairs (Defra), the Biotechnology and Biological Sciences Research Council (BBSRC), the Department of Health, the Department of Agriculture and Rural Development, Northern Ireland (DARD), FSA Scotland, FSA Wales, FSA Northern Ireland, Scottish Executive Environment and Rural Affairs Department (SEERAD) and Scottish Executive Department of Health (SEDH).
- 2.2 The MSFFG project database² was used to identify projects for inclusion in this report. The *Campylobacter* related projects were found by searching the database for Organism 'Campylobacter'. Projects which were completed by the date of the 1999 MSFFG *Campylobacter* report (taken here to be 1st August 1999) were not included. Projects initiated after June 2003 were also not included, other than to indicate that relevant work has been funded. This gave a total of 89 projects. Two additional projects were added to the list based on their project details (BBSRC projects 0455 and 0891). A full list of the projects used for this report is attached at Appendix 2.
- 2.3 Studentships have been omitted from consideration.
- 2.4 Research funded by other agencies, including the Wellcome Trust, Royal Society, NHS Scotland, Health Protection Agency (HPA) and the Medical Research Council (MRC), as well as international research is not included within the body of the report, but a summary of research funded through these bodies is given in section 4 (paragraphs 12-17) below.

² The MSFFG maintains a database containing information about research projects in the area of the microbiological safety of food that are funded by the members of the MSFFG. Members of the Group provide the project information from their respective project record systems.

3. SIGNIFICANT TRENDS IN RECENT RESEARCH

- 3.1 During the past four years, there have been a number of significant trends in *Campylobacter* research, as reflected in the projects awarded by MSFFG members and the scientific progress made as a consequence. Such progress is paralleled by similar trends throughout the international *Campylobacter* research community, although different funding agencies have different priorities.

New techniques for detection and classification

- 3.2 A number of new or improved techniques for the rapid identification of *Campylobacter* strains have been, or are, in development. Several of these (**Defra OZ0604, FSA B09002, FSA B03011, and FSA B03014**) use molecular biology techniques and approaches. Within these project reports, there is no consensus as to which of the methods are the most appropriate for use in detecting and analysing *Campylobacter* strains. However, at the CampyUK 2002/3 meeting, it was concluded that the method of choice for genotype classification should be Multi Locus Sequence Typing (MLST, see paragraph 5.2.4 below) and a number of studies are now using this technique (CampyUK2002/3 (2003)).

Use of molecular genetics

- 3.3 Molecular biology has become a major tool in *Campylobacter* research. In part this stems from the completion of the genome sequence of *Campylobacter jejuni* (Parkhill *et al* (2000)) and the development of a variety of research tools such as microarrays based on the genome sequence. Significant funding for this work has come from the Wellcome Trust. As a result, and in keeping with the vast majority of current microbiological research, tools and techniques such as expression studies using microarrays (**BBSRC D11362**), genome diversity (**BBSRC D13925**), proteomics (**BBSRC BFP11390**) a signature tagged mutagenesis (STM) library (**Defra VF0101**) and mutant library construction (**BBSRC EGA16166/67/74**) are being developed and used. These are relevant to a wide variety of aspects of *Campylobacter* research, both basic and applied.

Understanding the physiology of *Campylobacter*

- 3.4 Research in this area is beginning to lead to an understanding of the physiology of *C.jejuni* and other campylobacters. In particular, understanding of the interaction between the organism and oxygen has increased in recent years (eg **BBSRC BFP 11346, D18189, D14250, BFP 11294, 4301055, 2258371**) and there is an emerging picture of an organism which has a variety of mechanisms geared at both protecting it from high levels of oxygen (as are encountered in the food chain) and

respiring even under very low oxygen levels (as are encountered in the intestinal tract).

4. RESEARCH FUNDED BY OTHER ORGANISATIONS

Within the UK

- 4.1 Within the UK, the majority of *Campylobacter* research is currently funded by the BBSRC, Defra and the FSA. There is no current research of significance funded by the MRC (no projects recorded in their current public databases). The Wellcome Trust has funded the sequencing of the genome of *Campylobacter jejuni* and the Bacterial Microarray Group at St George's³ as well as some research into Guillain-Barré syndrome. In 2002 the Trust supported two research projects, one on *Campylobacter* classification and the other on the molecular biology and virulence of the organism.

Within the USA

- 4.2 The National Institutes of Health (NIH) are supporting a number of *Campylobacter* research projects. These can be found through their grants database CRISP⁴. There are at least 30 *Campylobacter* research projects recorded in the database as being active since 1999 (some with several components of the grant and more than one Principal Investigator). The abstracts of these grants provide an overview of the research funded in this area. All but two or three of the projects cover research on *Campylobacter* in man. Approximately half address issues of virulence, pathogenicity and host interaction with the organism. In particular, investigation of the mechanisms of virulence are common. A significant number of the projects exploit the genome sequence and there are several projects on other conditions in man with which *Campylobacter* is associated (eg Guillain-Barré syndrome).
- 4.3 A number of US bodies supporting agricultural research have a combined database for the Food Safety Research Information Office⁵. This database records approximately 60 separate *Campylobacter* research projects as being active during the period of this report. These projects cover the same broad range of research areas which are addressed through the MSFFG, with small numbers of projects in each area. The area receiving the most attention is the resistance of campylobacters to antimicrobial agents, encompassing approximately 20% of the funded projects. (Pathogenicity issues, detection and risk assessment are the next most frequently funded areas). There appear to be no projects addressing *Campylobacter* classification. There are

³ <http://bugs.sghms.ac.uk/index.php>

⁴ <http://crisp.cit.nih.gov/>

⁵ <http://www.nal.usda.gov/fsrio/index.htm>

two projects to develop poultry vaccines against *Campylobacter*.

Within the EU

- 4.4 The CORDIS database⁶ records about ten EU funded projects on *Campylobacter*. Again, the range of research areas is very broad, with no clear focus in a particular area. One of the two projects developing molecular subtyping of campylobacters is the consortium CAMPYNET⁷ (eg see paragraph 5.2.8).

Summary

- 4.5 The overviews described in paragraphs 4.1 to 0 are undoubtedly incomplete. In particular, the CORDIS database does not cover nationally funded research in the EU. There will also be other research funded within the USA. No attempt has been made to identify industry-sponsored *Campylobacter* research. Finally, without more extensive checking of databases, it is probable that relevant projects have not been identified. However, these limitations aside, the emerging picture is one of relatively limited *Campylobacter* research outside the UK.

⁶ <http://www.cordis.lu/en/home.html>

⁷ <http://campynet.vetinst.dk>

MSFFG FUNDED *CAMPYLOBACTER* RESEARCH FROM 1999 TO 2003

5. DETECTION, DIFFERENTIATION AND DIAGNOSIS

5.1 The area of detection, differentiation and diagnosis is broad. It addresses the need to develop methods for identifying those strains of *Campylobacter* which are of clinical relevance, approaches to subtyping required so that epidemiological studies can be undertaken, methods for isolating the organism and for detecting the presence of viable non-culturable (VNC) forms of *Campylobacter*. It covers research undertaken in man, animals (in particular poultry) and foods.

5.2 Overview of current research

Detection

5.2.1 There are projects to develop rapid PCR assays for the detection and differentiation of *Campylobacter* including for use in food and related environments (TaqMan PCR assay, **FSA B09002**) and in public health contexts (PCR/Bi probe test, **FSA B03011**). (There are existing PCR assays for *Campylobacter* but these involve gel electrophoresis or ELISAs.) Both new PCR-based assays could detect and differentiate between the species of *Campylobacter* presented to the assay. In a small scale field trial, the PCR/Bi probe test was able to detect *Campylobacter* in all samples testing positive by a PCR/ELISA method, making the new method more sensitive than conventional culture identification and more rapid than the PCR/ELISA test (**B03011**). The TaqMan PCR assay (**B09002**) was used on *Campylobacter* contaminated food samples after culture and was found to be effective. There was no recorded comparison with existing assays, although the researchers regarded the assay as a model for use with other food-borne pathogens. Both these projects have used genome sequence information to develop appropriate unique primers for the detection of particular species of *Campylobacter*.

5.2.2 A further project (**Defra OZ0608**) has evaluated a variety of culture methods for *Campylobacter*, but no definitive outcome is reported. In addition, research to compare isolation methods as part of a study of campylobacters from man and poultry (**DARD 0117**) led to a standardised protocol for the culture of *Campylobacter* found in raw poultry.

5.2.3 There is some current research on detection methods for culturable and non-culturable campylobacters (**DARD/InvestNI ST200**).

Classification at the genomic level

- 5.2.4 Multi locus sequence typing (MLST) uses the variations in sequence fragments of a small number (seven) of 'housekeeping' genes of campylobacters to classify isolates of the organism. It has the advantage of being based on characteristics of the organism which are independent of time or testing lab: the benefits of this have led to MLST schemes for a number of different pathogens. Two possible primer sets (for the sequence fragments) were compared (**Defra OZ 0604**), and it was found that there were no significant differences between the two methods. The scheme of Maiden *et al* (1998) has been used to establish a database for the identification of *Campylobacter* types (Dingle *et al* (2001))^{8,9}. This database provides a record of over 800 different allelic isolates (ie *Campylobacter* types which are differentiated on the basis of different sequence patterns using the MLST scheme) and is a significant resource available for international use. Other projects are now actively using this approach to the classification of *Campylobacter* types (eg **Defra OZ0607**).
- 5.2.5 Several projects have been used to develop approaches to determining genomic variation in *Campylobacter*. One technique using genome sequence data that is being used to explore *Campylobacter* genomic variation is Amplified Fragment Length Polymorphism (AFLP) (**FSA B03014, Defra OZ0605**). This method is also regarded as providing more sensitive analytical methods than the existing culture-based (phenotypic) ones. AFLP was used to consider whether there was correlation between this assay and the existing serotyping and phage typing approach (**FSA B03014**). As with the effort to relate patterns of genotypic instability with serotype/phage type, no clear correlation was found. However, it was argued that conventional typing was based on too small a gene set, and that AFLP would provide a more accurate technique.
- 5.2.6 Using AFLP, one project (**Defra OZ0605**) found that there was a high level of genomic instability in *Campylobacter* which did not have any correlation with serotype variation. In addition, the variation was too great to be useful in identifying the food chain/animal source of a specific human infection. The degree of genotypic variation is supported by the findings of the genome sequence work (Parkhill *et al.*(2000)) which in turn is the basis of the project to explore the genomic variation of *Campylobacter jejuni* (**BBSRC D13925**).
- 5.2.7 Ribotyping has also been developed and used to explore the variation in *Campylobacter* genotypes in farms (**DARD/InvestNI ST200**). It was concluded that the methodology could be used as an effective

⁸ <http://campylobacter.mlst.net/>

⁹ Reference to paper based on work funded by Defra OZ 0604

molecular typing approach for *Campylobacter*.

- 5.2.8 Characterisation of *Campylobacter* strains using a number of different methods, PCR-RFLP of flagellin, AFLP, RAPD and PFGE has been carried out in collaboration with EU researchers through the CAMPYNET¹⁰ (**DARD 9641**). Standardised methods were designed for all assays, but these were not necessarily transferable between laboratories. It was recognised by the project that there were further advances being made in *Campylobacter* typing, such as MLST, which could overcome some of these issues.
- 5.2.9 The development of the MLST database (see 15 above, **Defra OZ0604**) has enabled the classification of over 800 different *Campylobacter* profiles (distinct MLST patterns). This is being used to assess field samples from farm poultry and wild birds. The database is likely to have utility in assessing the source of different strains of *Campylobacter* from a range of situations. Comparisons of campylobacters using this method and the associated database with an alternative scheme have supported the repeated observation that the *C. jejuni* population is very diverse. Furthermore, there did not appear to be specific human or veterinary strains (**Defra OZ0602**) and some human-associated types of *C. jejuni* were present in a wide range of samples including livestock, wildlife, water and soil (**Defra VF0201**).
- 5.2.10 There may be as yet unidentified opportunities for developing novel identification methods for both industrial and clinical applications based on genomic or proteomic information. An example is the recently initiated project (**FSA B14012**) in which rapid sequence based typing will be used as the basis for identifying *C. jejuni* strains and investigating the associated epidemiology.

Standardisation of testing

- 5.2.11 The opportunities to develop new tests for detecting and classifying *Campylobacter* are extensive. However, for any of these methods to be introduced to the food industry, or the hospitals, they have to be better than those already in place. Some work has been done to understand what testing is undertaken within the food industry. It was found that the sampling and analytical methods in the food industry were varied (**FSA M01017**) but that the most commonly used approach for detection was culture of the organisms (**FSA B09005**). There was some evidence of resistance to change, associated with cost and need, but also with the customer acceptability.
- 5.2.12 A significant study using several different genotyping techniques investigated the various *Campylobacter* types present in poultry flocks and during processing (**FSA B03003**). The project used *fla*-typing followed by PFGE and AFLP to confirm the typing results. Consistent

¹⁰ <http://campynet.vetinst.dk>

results in terms of classifying individual isolates using *fla*-typing and PFGE were reported. A study of stored infectious intestinal disease *Campylobacter* samples concluded that molecular techniques, in particular PCR detection of the hippuricase gene, should be the method of choice if no further subtyping was available (**FSA B10002**).

5.3 Assessment and identification of gaps

Gaps identified in the 1999 MSFFG *Campylobacter* report

5.3.1 In the 1999 MSFFG *Campylobacter* report, there was recognition that real progress had been made in research in the detection, diagnosis and differentiation of *Campylobacter* species. However, a number of areas for further research were identified. These included:

5.3.2 In the area of detection:

- Communication between researchers was needed to ensure that validated molecular methods were 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 was necessary
- The development of a rapid method for the detection of contaminated poultry flocks at or before slaughter was required.

5.3.3 In the area of differentiation there was a need for communication between researchers to ensure that subtyping methods were well defined so that internationally acceptable typing schemes could be established.

5.3.4 There was at the time no unified approach to *Campylobacter* subtyping. A number of research groups had 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 included PFGE, phage typing and resistotyping. However, there remained a gap in communicating this important message to the research community so that these methods could be introduced as the standard approach to *Campylobacter* subtyping.

5.3.5 With respect to diagnosis of human disease, there was a need for non-invasive diagnostic tests, e.g. salivary based tests.

Assessment of progress and identification of current gaps in research

- 5.3.6 In line with the identified needs in the 1999 MSFFG *Campylobacter* report, significant work has been undertaken in the development of assays and methods for the classification of campylobacters, in particular based on genotypic information. Of the classification methods developed the MLST typing method appears to be the most appropriate and robust currently available and is increasingly the method of choice. Standardised methods for preparation of DNA for MLST sequencing are available (<http://campylobacter.mlst.net>). The outstanding gap may be that of communication within the research community and between it and the funding bodies to ensure that there is formal confirmation of the selection of the MLST as the current classification scheme of choice for all current and future *Campylobacter* projects. The MSFFG may also need to know the views of the European and wider international research community on this subject.
- 5.3.7 However, there may still be a need for further standardisation of sampling and assays for detecting campylobacters in a variety of environmental, animal and human contexts.
- 5.3.8 There is a need for the MLST methods to be applied widely across the range of *Campylobacter* research. This should assist in addressing gaps in understanding of the epidemiology and pathogenicity of the organisms.
- 5.3.9 There may also be a gap in research to ensure that detection and MLST classification methods are appropriate for use by testing laboratories outside of the research community.
- 5.3.10 There is still no progress in developing immunological tests to determine whether humans have been exposed to campylobacters.
- 5.3.11 Substantial progress has been made in developing systems for classifying campylobacters. These, and much research into the variability of the organism, have emphasised that there is no single strain that could be regarded as fully representative of *Campylobacter spp.* It is therefore important that when selecting a strain for research, the reasons for the selection should be clear. It should also be stressed that the diversity of campylobacters should be recognised appropriately in research projects.

6. MICROBIAL PHYSIOLOGY AND GENETICS

6.1 The study of the physiology and genetics of *C. jejuni* aims to provide for a basic understanding of how the organism functions and why it responds in a given manner to a particular environment or set of circumstances. This has been greatly enhanced in recent years through knowledge of the *C. jejuni* genome sequence, which is the basis for research which will lead to the identification of the function of many, and eventually all, of the genes within the bacterium. Understanding these functions provides potential targets for intervention, both in man and in other situations, including poultry and the environment.

6.2 Overview of current research

Exploiting the genome sequence

6.2.1 During the last four years, several major projects have been funded with the intention to exploit the *C. jejuni* genome sequence (**FSA B03014, BBSRC D13925, BFP11362, 4301055, 4311209**). These have provided resources for research such as *Campylobacter* expression and genomic microarrays, which are currently in use by the research community. For example, microarrays were developed for gene expression studies and by the end of 2002 around 800 copies of these had been duplicated and used to study *C. jejuni* transcription under a range of physiological conditions (**BFP 11362**). Expression studies have led to increased understanding of flagellin and capsule expression amongst other facets of *C. jejuni* biology (**BFP 11362**).

6.2.2 Microarrays can be used for expression studies and for genomic studies. In order to facilitate the latter, a DNA microarray including the genes from the sequenced strain of *C. jejuni* and more than 100 sequences for several further strains of diverse origin has been constructed (**D13925**). This is being used to study strain variation in relation to disease and host source.

6.2.3 Most recently, work has been funded to delete and tag every gene from *C. jejuni* and thereby to determine the function of each gene (**BBSRC EGA 16166/67/74**). The initial priorities are genes of unknown function which are specific to *Campylobacter* and genes expressing membrane associated polypeptides.

Proteomic analysis of *C. jejuni*

6.2.4 Proteomic analysis is an increasingly important component of genomic research, and methods and analysis for proteomic analysis of *C. jejuni* have been devised (**BBSRC BFP11390/91/92**). Using these, proteomic analysis of the organism under a variety of growth conditions has been undertaken. Comparison of the proteome of the sequenced

strain (NCTC11168) with strain NCTC 8116 (the strain used at the Institute of Food Research) as well as with a number of mutants has also been undertaken. This has shown that there are considerable differences in the protein pattern of the two strains, which heterogeneity is not necessarily reflected at the genome sequence level. This may prove important in relation to the interaction between the pathogen and its host.

- 6.2.5 One direct outcome of the proteomic analysis was the finding that *C. jejuni* has a novel N-linked glycosylation system which is now subject to further investigation (**BBSRC D15819**). Initially two surface glycoproteins were identified. Subsequently, a further 30 glycoproteins with N-linked glycosylation have been identified. It is anticipated that these will contribute to the interaction between the pathogen and host cells and immune system during infection. As an indicator of this, a mutant deficient in the glycosylation pathway was found to be less able to adhere to intestinal cells, or to colonise chickens. It is noted that the glycan structure of the glycoproteins is conserved between the various *C. jejuni* strains examined. This is in contrast with many other surface determinants, which vary extensively between strains.

Environmental effects on gene expression

- 6.2.6 Many bacteria use two component regulatory (TCR) systems to respond to specific environmental signals and stimuli and thus to regulate gene expression. Two families of proteins are involved, membrane histidine kinase sensor proteins and cytoplasmic DNA-binding response regulators. Together these transmit environmental signals to bacterial response mechanisms. Five such pairs of proteins have been identified in *C. jejuni* as well as several other unlinked proteins of each family. Creation of mutants and expression of these in a variety of growth conditions has enabled assessment of the possible gene function. This is being followed by proteomic analysis of the TCR mutants which should inform understanding of the regulation of *C. jejuni* environmental responses and control (**BFP 11390/01/02**).
- 6.2.7 A specific, novel TCR system was identified (Bras *et al* (1999)) and subsequently further characterisation undertaken (**BBSRC D09207**) in order to determine details of the environmental response of the RacRS system and of the interactions of the genes in the regulon. Data so far suggest that the RacRS system does respond to both heat and iron levels in the growth environment, and that it controls a number of genes including some involved in amino acid metabolism.
- 6.2.8 The proteomic analysis of *C. jejuni* has also led to a greater understanding of the flagellar structure for the organism (**BFP11390/91/92**). Virulence of the organism is also related to the production of chemotactic proteins, and again the proteomic analysis is contributing to the understanding of this, including the regulation of chemotaxis. Regulatory components of these pathways are potential

targets for intervention in preventing intestinal colonisation of chickens by the organism.

Biochemistry and physiology of *C. jejuni*

- 6.2.9 *C. jejuni* is microaerophilic and there are several projects based around research in this area (**BBSRC BFP11346, BFP 11294, D14520, 3258371**) including a number which have been recently funded and from which there are as yet little or no data (**BBSRC D18368, D18189, D19661/2**). The importance of understanding the underlying reasons and mechanisms of the poor oxygen tolerance of *C. jejuni* will help inform understanding of the invasiveness and pathogenicity of *C. jejuni*. It is also relevant since, whilst the organism respire and grows optimally under microaerophilic conditions (5-10% oxygen) it encounters higher levels of oxygen in the food chain and survives these (**BFP 11346**). In addition, the genome sequence suggests that the organism has few regulatory mechanisms for providing response to stress tolerance, which makes the respiratory versatility of the organism more difficult to explain.
- 6.2.10 Two oxidases, involved in oxidase-catalysed oxygen uptake, have been identified in *C. jejuni*, one of which was predicted from the genome sequence (**BFP11346**). In addition, two haemoglobins have been found, and synthesis of one of these is up-regulated by nitrosative stress (for example, the presence of nitric oxide). This finding has led to further research into the ability of *C. jejuni* to resist nitric oxide and nitrosative stresses (**BBSRC D18084/18368**).
- 6.2.11 Although microaerophilic, *C. jejuni* does have an essential requirement for oxygen to act as electron acceptor in respiration (**D18189, D14250**). In strictly anaerobic conditions with a variety of possible electron acceptors there was insignificant growth of the organism, suggesting that, despite the presence of several oxygen labile enzymes, there is an essential step within *C. jejuni* metabolism for which oxygen is necessary. Analysis of the genome of *C. jejuni* suggested that the likely candidate was an enzyme required in DNA synthesis, namely ribonucleotide reductase, for which there was no alternative and which needed oxygen in order to function. Some electron microscopy was undertaken which supported this conclusion (**BFP 11294**) and further work is proposed (**D18189**).
- 6.2.12 Given that *C. jejuni* has highly oxygen labile essential enzymes, but is also an obligate aerobe, the organism must have evolved mechanisms for addressing these competing issues. Research has shown (**BBSRC 4301055, 3258371**) that there is at least one system playing an important role in defence against oxidative stress and that the regulation of iron metabolism is similarly a component of the organism's defences. In addition, analysis of the proteome of *C. jejuni* grown under oxygen limitation and microaerobic conditions shows that there are several proteins from the TCA cycle and oxidative stress

resistance which are down-regulated in oxygen limited conditions, and at least one enzyme involved in response to anaerobiosis which is up-regulated in the same conditions (**D14250**).

6.2.13 Research into the carbon metabolism of *C. jejuni* has shown that serine is possibly the most significant carbon source, with an oxygen labile serine dehydratase as a component of the catabolic pathway (**BFP11294**). Importantly, by creation of an appropriate mutant, the researchers were able to show that the ability to catabolise serine is vital to the colonisation of chickens. The *in vivo* requirement for serine is possibly linked to the conversion of serine to pyruvate which is the primary compound feeding into the TCA cycle. The enzymes¹¹ involved in the conversion of pyruvate into acetyl CoA are very similar to those for the same step in *Helicobacter pylori*, which have been found to be extremely oxygen labile.

6.2.14 Equally, *C. jejuni* has been found to be unable to utilise glucose as a carbon source and therefore requires synthesis of glucose through gluconeogenesis. Study of the gluconeogenic pathway using mutants revealed at least one essential gene¹² and two further genes which appeared to be necessary for successful colonisation of chickens¹³ (**BFP11294**).

Detection of viable non-culturable (VNC) forms

6.2.15 There are no reports making any significant comments on this subject in the relevant projects in the MSFFG project database.

6.3 Assessment and identification of gaps

Gaps identified in 1999 MSFFG *Campylobacter* report

6.3.1 In the 1999 MSFFG *Campylobacter* report, it was recognised that gaps in knowledge relating to the physiology of *Campylobacter* (in terms of the effect of temperature, pH, and water activity) were being addressed through funded research. However, there remained a need to increase knowledge of:

- The organism's metabolism, the physiological basis for its microaerophilic nature and its requirement for elevated levels of carbon dioxide for growth.
- The viable non-culturable (VNC) state in *Campylobacter* which might be a response to certain environmental conditions whereas the

¹¹ The enzymes concerned are flavodoxin/ferrodoxin dependent 2-oxoacid oxidoreductases, as opposed to the more commonly occurring, and not oxygen labile, NAD-dependent dehydrogenases.

¹² Phosphoenolpyruvate carboxykinase, *pckA*

¹³ Pyruvate carboxylase *pycA* and pyruvate kinase, *pyk*

pathogenic significance of VNCs was not clear.

- 6.3.2 Researchers were also encouraged to make the best use of the data arising from the *Campylobacter* genome project when investigating the organism's physiology and metabolism.

Assessment of progress and identification of current gaps in research

- 6.3.3 There has been substantial progress in understanding of the physiology of *C. jejuni*, in particular in the areas of molecular biology and research which exploits the *C. jejuni* genome sequence. There continues to be a need for further understanding of the physiology of the organism, particular in relation to its pathogenicity and invasiveness.
- 6.3.4 Equally, the necessary coherent understanding of the physiology of *C. jejuni*, and other campylobacters, has yet to emerge.
- 6.3.5 There is some overlap between research by different groups and the collaborations which are increasingly in place should be encouraged.
- 6.3.6 There remains an absence of research on the viable non-culturable forms of campylobacters. However, it has been concluded that currently there is no apparent need to proceed with research in this area, until it is clear that VNCs are of importance.

7. PATHOGENESIS

- 7.1 The pathogenicity of any infectious agent is a consequence of its repertoire of genes and proteins, and the interaction of the organism with its environment, in particular the host. The virulence of any given pathogen will be affected by these factors, which may vary between strains of pathogen, and between different hosts. In the case of *C. jejuni*, there is ample evidence of extensive variation between strains in terms of virulence, amongst other features. These issues are addressed through research into the pathogenicity of campylobacters.
- 7.2 For a bacterium such as *Campylobacter*, which is capable of colonising a host without causing disease, research into pathogenesis also addresses the processes underlying this ability. Thus pathogenesis research includes work undertaken in chickens, as well as consideration of the interactions between *Campylobacter* and man.

7.3 Overview of current research

Basic research

7.3.1 Using recent genetic manipulation methods for *C. jejuni*, at least one gene has been found which may be involved in invasiveness (**FSA B01005**). Using proteomic techniques, glycosylated surface proteins in *C. jejuni* have been identified and further work is under way, which may contribute to understanding of host pathogen interaction (**BBSRC D15819**).

Colonisation of chickens

7.3.2 Basic research with *C. jejuni* has revealed a number of factors which appear to influence the ability of the organism to colonise chickens. For example, serine appears to be a crucial carbon source for *C. jejuni in vivo*, and there are specific enzymes from within pyruvate metabolism which are also needed for successful colonisation of chickens by the pathogen (**BBSRC BFP11294**) (see paragraphs 6.2.13 and 6.2.14 above). It is possible that in the absence of serine, and the necessary pathway to ensure a supply of pyruvate to feed the TCA cycle, *C. jejuni* would not be able to colonise chickens.

7.3.3 Work is ongoing to derive *C. jejuni* mutants which have altered colonising abilities in order to identify genes which are essential to bacterial colonisation of avian gut (**Defra OZ0606**). At this stage, a number of mutants have been identified, but no clear patterns identifying specific mutants as having altered colonisation properties have yet been observed.

7.3.4 *C. jejuni* mutants have been generated using signature tagged mutagenesis (STM) (**Defra VF0101**) and strains with the mutation in non-essential genes have been introduced into chickens in order to identify those mutants in which a gene required for colonisation in chickens had been knocked out. The results so far have found that, after the introduction of a mixture of *C. jejuni* strains to chickens¹⁴, the majority of the strains are eliminated leaving only one (not the same one in each experiment) to colonise the chicken. Similar work is being done on new-born chicks to investigate this phenomenon.

7.3.5 A recent project has been funded to examine mechanisms by which *C. jejuni* colonises chickens but no data are yet available (**BBSRC**

¹⁴ The experimental method used involves mixing cultures of many different mutants of *C. jejuni*. This mixture is then analysed on a specific genomic array to determine which mutants are present. Chickens are infected and at an appropriate time the surviving *Campylobacter* strains are extracted. These are then assessed in the same manner as the 'input' mutant mixture, to determine which mutants are still present. The method is such that any mutant in which the mutation was within a gene essential for colonisation in chickens will be absent from the 'output' strains.

0941).

Pathogenicity in man

- 7.3.6 In order to examine the ability of campylobacters from chickens to invade humans, over 100 poultry isolates were screened in a tissue culture model of invasion. It was found that only 2% of the isolates were highly invasive in the model. In contrast, using the same model, 20% of isolates from human disease were highly invasive (**FSA B01005**). Although there are significant issues still to be addressed, the results fit with the general variability of campylobacters as pathogens. So too do results obtained when determining the invasiveness of *Campylobacter* strains and their ability to cross epithelial monolayers in culture.
- 7.3.7 It has been suggested that the processing of chicken containing campylobacters may affect the invasiveness of the organisms, either by forcing adaptation or selection of more virulent forms. Results showed that processing might, on very rare occasions, have such an effect but that in general this was not the case (**FSA B01005**).
- 7.3.8 Using genomic and other techniques, such as the MLST scheme, a major study is comparing pathogenic and non-pathogenic strains of *C. jejuni* in order to identify bacterial factors which may be associated with virulence (**Defra OZ0607**). Preliminary conclusions have suggested that for the hyperinvasive strain investigated, reduced motility did not correlate with reduced invasiveness. The same project has also explored (with project **Defra OZ0602**) the role of cytolethal distending toxin (CDT) in causing human enteric disease. The genes of toxin-negative mutants are being investigated to identify the location and nature of specific mutations and their significance in toxin production. One of these CDT mutants was obtained from a patient with a history of enteric disease prior to bacteraemia. This may suggest that CDT is not essential for inducing enteric disease (**OZ0602**). Further data from this project, presented at the CampyUK 2002/3 meeting (CampyUK2002/3 (2003), were summarised as indicating that CDT is not always necessary for *C. jejuni* pathogenesis in man or colonisation of poultry. However, CDT is expressed *in vivo* during human infection and its presence leads to antibody production against antigenic sites which are not recognised by the chicken immune system. These data suggest that the toxin is both expressed during human infection and is immunogenic.
- 7.3.9 Parallel questions are being explored by investigating whether all *Campylobacter* strains are capable of causing disease and whether there is a genetic basis for the different virulence properties or disease outcomes seen in humans (**BBSRC 3258371/411209**).
- 7.3.10 Other mechanisms by which *C. jejuni* triggers inflammation and fluid loss in (human) gastrointestinal mucosa are currently being

investigated. An initial focus is on bacterial cell surface proteins and toxins (**BBSRC D14376**). Early results have shown that a model enterocyte host cell line shows significant changes in gene expression in response to *C. jejuni* infection. In addition, evidence has been found that infection of these cells by *C. jejuni* inhibits their ability to absorb fluid, which is a possible mechanism for occurrence of diarrhoea during infection. The role of the flagella, including molecular modes of action, in pathogenicity are also being investigated (**VF0101**).

7.4 Assessment and identification of gaps

Gaps identified in 1999 MSFFG *Campylobacter* report

- 7.4.1 In the 1999 MSFFG *Campylobacter* report, it was recognised that there were significant gaps in the understanding of the pathogenic mechanisms of *Campylobacter*. It was recognised that the full extent of the variation in the virulence of *Campylobacter* strains was yet to be determined. In addition, the mechanisms by which campylobacters caused disease were still not established. In particular it remained unclear why campylobacters caused disease in humans and in some animals but not in other animals
- 7.4.2 A model of *Campylobacter* infection had been developed which might become, once fully established, an extremely useful resource for understanding the pathogenic mechanisms of *Campylobacter*.
- 7.4.3 Additional gaps in funded research were identified as follows:
- Latest research had called into question the validity of *in vitro* assays for monitoring pathogenicity as they appeared to monitor different effects from *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) remained unclear and further research was needed to clarify the clinical significance of toxin production
 - It was not clear whether VNC forms represented a food safety problem. Funded research suggested these forms lacked the ability to colonise the gut, but results to the contrary had been published. Further research and use of the animal model for *Campylobacter* might clarify this issue
 - There was a need to establish and validate an animal model which was suitable for studying *Campylobacter* infections.

Assessment of progress and identification of current gaps in research

- 7.4.4 Within the research effort described in this report, the understanding of the pathogenicity of campylobacters, and the mechanisms of colonisation of chickens does not appear to have made great progress. To some extent this is compounded by the lack of progress in understanding of the epidemiology of campylobacters (see section 8 below). Many of the issues identified in the 1999 MSFFG *Campylobacter* report have not, in fact, been addressed, or have been so with only limited success.
- 7.4.5 There is recently funded research which is seeking to consider some of the issues raised in the 1999 MSFFG *Campylobacter* report, including virulence factors and the role of CDT (**Defra OZ0607, OZ0602, BBSRC 3258371/411209**). However, there are still gaps in research into the wider application of the development of the MLST scheme and the association of phenotypic properties with genotypic information.
- 7.4.6 At the CampyUK2002/3 meeting, the general consensus was that the reasons for illness in man caused by campylobacters were toxin production, invasion and inflammation and that the variation in pathogenicity was likely to result from the interaction between host and pathogen. In particular, host response to the organism was regarded as being paramount in determining the pathology of the organism and that there is no real evidence of specific *Campylobacter* strains being associated with illness, in contrast to the situation with *Escherichia coli* (CampyUK2002/3 (2003)). There appears to be little research on host-pathogen interactions and host response to invasion.
- 7.4.7 There is still a lack of adequate validated models of colonisation and pathogenicity of *Campylobacter* in man and poultry.
- 7.4.8 The lack of any immunology research associated with *Campylobacter* infections was highlighted by the MSFFG. This situation is partially due to the known lack of immune response to enteric infections. However, it was recognised that there could be situations in which a greater understanding of the immune response to the organism would be valuable. In particular, this would be relevant when considering the possibilities of food industry workers introducing infection to others.

8. EPIDEMIOLOGY

8.1 Studies of the epidemiology of *Campylobacter* need to address issues of human infection and the source(s) of infection. There is a need to determine which strains and subtypes of *Campylobacter* can be found in which hosts, and what patterns of occurrence of these strains there may be. Epidemiological studies therefore explore the presence of campylobacters in a wide range of potential host organisms, whilst focussing primarily on poultry and man. As a component of this, studies of the food chain, and poultry handling and producing processes are necessary, alongside studies of the occurrence of campylobacters in a range of other environments.

8.2 Overview of current research

Campylobacter in the food chain

- 8.2.1 It is presumed that a principal source of campylobacters leading to infection of mankind is poultry (The Strategy for the Reduction of *Campylobacter* in Broiler Flocks (2003)). Research has been undertaken in recent years to explore the extent to which *Campylobacter* is associated with the agricultural, poultry and food industry.
- 8.2.2 For example, levels of pathogens present in abattoir wastes for application to agricultural land were measured. *C. jejuni* was the most frequently identified bacterial pathogen, although the level of occurrence, in 5.7% of samples, was low (**FSA B05008**). These levels were such that questions of seasonal or locational variation could not be explored. Similar work sampling farm slurries found that 22% of samples contained *Campylobacter* (**FSA B05003-4**). This research also determined that in the slurries spread onto land, the numbers of any pathogen fell to below detectable levels within 32 days. No specific comment is made about *Campylobacter*, but given the lability of the organism and its demanding physiological requirements, it would be likely that it survived for less time than this. Analysis of the risks associated with the application of manures and abattoir wastes to agricultural land indicated that a significant step in reducing the risk of transferring pathogens from manures to crops or grazing livestock would be the storage of the slurries for appropriate prescribed minimum periods (**FSA B17002**).
- 8.2.3 Analysis and modelling of farm and abattoir waste and the risks of application of these to agricultural land has led to the finding that storage of the slurries for appropriate prescribed minimum periods of time would provide an effective barrier to transfer of pathogens into the food chain (**FSA B17002**). However, the study acknowledges that more information is required about pathogen decay under the storage

conditions. Similar conclusions are reached by a study of a wide range of pathogens in livestock manures, along with the recognition that there would be cost implications (**Defra WA0656**).

- 8.2.4 Although the basic assumption is that the principal risk to man from campylobacters is exposure through infected poultry, there is also research to determine the spread of food-borne pathogens, including *Campylobacter*, through cattle. In particular, the point of entry of livestock into the abattoir has been identified as a critical control point (**FSA M01013**).
- 8.2.5 Comparison of the varieties of isolates of *C. jejuni* in a range of samples, including livestock, water, soil and wildlife has found human associated types in all samples (**Defra VF0201**). A Northern Ireland study found that there were shared *Campylobacter* genotypes in isolates from poultry, other livestock and the environment (**DARD/InvestNI ST200**). Other studies are examining the relationship between campylobacters in the environment and the initial isolation of campylobacters from broilers (**Defra OZ0608**).
- 8.2.6 Linking the management of commercial broiler flocks with that of other species, it has been found that broiler staff working with other farmed species, coupled with an absence of appropriate biosecurity precautions, represent a significant route for the introduction of *Campylobacter* to chickens. The process of partial depopulation of the flocks (thinning), as identified in The Strategy for the Reduction of *Campylobacter* in Broiler Flocks (2003) also provides a significant route for the introduction of *Campylobacter* infections (**ST200**).
- 8.2.7 An extensive study of poultry production and processing using several different *Campylobacter* typing techniques identified both the environment and carry-over of strains from a previous flock as possible routes for contamination (**FSA B03003**) although carry-over was found to be relatively infrequent. Study of campylobacter-negative flocks suggested that there were at least two factors contributing to the susceptibility of such flocks to infection: the presence of competitive gut flora in older birds and, possibly, the presence of maternal antibodies in young ones.
- 8.2.8 Examination of the management of poultry from farm through processing to packaging showed that the farm and processing plant environment tended to be campylobacter-positive even when the poultry flock being managed or processed were campylobacter-negative. This did not seem to be the case for the poultry house air: sampling only found the pathogen when the birds were campylobacter-positive. Processing plants, including the air, were found to be contaminated not only with the strains of *Campylobacter* from the study birds, but also with other strains, suggesting previous contamination (**FSA B03008**). It was also found that carcasses from *Campylobacter* negative flocks rapidly became contaminated during processing (**FSA**

B03003).

- 8.2.9 It was noted that flocks and birds could be colonised by up to three *Campylobacter* subtypes, and that the strains and subtypes colonising a given flock appeared to be unique to that flock (**B03008**). This should be considered alongside the findings that prior colonisation of the avian gut with one *Campylobacter* strain could completely exclude a subsequent challenge strain (**Defra OZ0603**).
- 8.2.10 Separately it was observed that transport crates arrived contaminated (**B03008**), which fits with the observations and concerns of the FSA strategy for the control of *Campylobacter* in chickens, in particular in relation to thinning (The Strategy for the Reduction of *Campylobacter* in Broiler Flocks (2003)).
- 8.2.11 A further possible source of *Campylobacter* leading to contamination of poultry is thought to be the water supplied to the broiler flocks. Investigation of the presence of *Campylobacter* spp in water found that *C. jejuni* was more likely to be found in running water and *C. coli* in natural still water (**Defra VF0201**). Other research found that over a three year period, 44% of farms investigated had water delivery systems contaminated with *Campylobacter* at some stage in the study (**FSA B03010**). The causes for this were unclear as the project reported that the cleaning regime for the delivery systems was effective and that no *Campylobacter* was found post-cleaning even when a contaminated flock had been present. Despite this result, improved water treatment was suggested.
- 8.2.12 Research has been undertaken to determine the frequency of contamination of raw chicken with *Campylobacter* (**FSA B08008, B18002**). It was found that overall the frequency of contamination of UK sourced raw chicken by *Campylobacter* was between 50% (**B18002**) and 76% (**B08008**) (Jorgensen *et al* (2002)). Where the species of *Campylobacter* was determined, it was found that three quarters were *C. jejuni* overall, although this was higher in Scotland (83%) and Northern Ireland. In the more recent study (**B18002**) a number of different *C. jejuni* serotypes and phage types were found. In a separate small study, no *Campylobacter* was found in sausages (**FSA B02013**).
- 8.2.13 The occurrence of *Campylobacter* in chickens and chicken pieces for retail sale was found to be higher in UK-produced whole chickens than those sourced from outside the UK (**FSA B18002**) but similar for fresh and frozen chicken portions.
- 8.2.14 There is also some research considering the possible presence of campylobacters in other components of the food chain. For example, 25% of faecal samples from beef abattoirs in Northern Ireland were found to contain campylobacters (**DARD 9723**). Similarly it was found that the prevalence of *Campylobacter* species in faecal material on

beef farms was 18%, which was taken as a measure of the general incidence of campylobacters (**FSA M01009**). In a study in Cheshire, 84% of the beef and dairy farms examined were found to have at least one animal positive for *C. jejuni*, with 22% of lactating cows also being positive for the organism (**Defra VF0201**). In a study of a small number of these farms, all were found to be positive for *Campylobacter* spp over a twelve month period, with *C. jejuni* being very common (**VF0201**).

8.2.15 Research into the occurrence of campylobacters in sheep at abattoirs found only low levels of the organisms (**FSA M01011**).

8.2.16 Samples from foods and from man in Northern Ireland are being analysed for the occurrence of *Campylobacter* strains. It is currently proposed that these will be classified using AFLP. At an early stage in the project it has been found that 28.8% of foodstuff samples carried campylobacters (**DARD R7105FOS**).

8.2.17 Research into the presence of *Campylobacter* in the kitchen was consistent with the physical fragility of the organism, with *Campylobacter* being unable to persist for more than four hours on kitchen surfaces. In addition, no *Campylobacter* spp were found in kitchen cloths. (**FSA B02016, B02015**).

Campylobacter in man

8.2.18 A study of stored *Campylobacter* isolates from intestinal infectious disease found little variation in the predominant subtypes of *C. jejuni* and *C. coli* found in association with the condition over the period that the samples had been collected (**FSA B10002**).

8.2.19 In contrast, a detailed study, using MLST and antigen sequence typing, has found that there is a high level of genetic and antigenic diversity in strains of *C. jejuni* isolated from man. However, the highly varied isolates could be grouped into a much smaller set of clonal complexes, where there was clear genotypic relatedness between the isolates. There did not appear to be consistent patterns of association between antigens and particular clonal complexes (**Defra OZ0604**). In the same vein, use of amplified fragment length polymorphism (AFLP) showed little correlation between this sensitive test and conventional serotyping and phage typing (**FSA B03014**).

8.2.20 Work to try to identify and understand causes of the genetic instability was inconclusive, with the implication that genetic instability in *Campylobacter* strains was so frequent that it would interfere with surveillance and could in practice prevent identification of the source of human infections (**Defra OZ0605**).

8.2.21 The pattern of isolation of *C. jejuni* from human samples in the north-west of England and Northern Ireland is being monitored (**FSA**

B03013). Alongside this, there is research to determine whether there are significant similarities between campylobacters causing human disease and those found in poultry in Northern Ireland (**DARD 0117**).

Future work

8.2.22 Projects aimed at addressing various aspects of *Campylobacter* epidemiology have been initiated (**FSA B14012, B14011**). Both will consider the frequency and seasonal variation of *C. jejuni* and seek to relate these to possible significant events, with **B14012** focussing on the north-west of England. As a component of this, *C. jejuni* strains will be identified using rapid sequence-based typing and the utility of this will be examined. The second project will continue the examination of the epidemiology of *C. jejuni*.

8.2.23 Separately, work will be undertaken to determine whether the health status of the flocks affects their resistance to *Campylobacter* and whether vaccination is a risk factor for colonisation with the organism (**FSA B15001**).

8.3 Assessment and identification of gaps

Gaps identified in 1999 MSFFG *Campylobacter* report

8.3.1 In the 1999 MSFFG *Campylobacter* report, various outstanding issues were identified. For example, there was a lack of knowledge as to the extent to which campylobacteriosis is food-borne. It was indicated that there was a need for a unified approach to *Campylobacter* subtyping to support the understanding of the epidemiology of *Campylobacter*. Gaps in knowledge were identified in the 1999 report as follows:

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

Assessment of progress and identification of current gaps in research

8.3.2 Research reported here has given a clearer picture of the extent to which *Campylobacter* infection is food-borne, with the evidence that 50% of chicken carcasses are contaminated with the pathogen. In addition, there has been progress in understanding the epidemiology of

Campylobacter in poultry, both through research funded by the MSFFG members and industry based research. Some of this work provides the basis of the strategy for the control of *Campylobacter* (The Strategy for the Reduction of *Campylobacter* in Broiler Flocks (2003)). However, there is still a lack of information on the source(s) of the introduction of campylobacters to any given poultry flock, although there is a study funded by Defra investigating the epidemiology of *Campylobacter* in poultry production which will identify and rank sources of infection (**Defra OZ0608**).

- 8.3.3 There remains a lack of information about the relative importance of the source of *Campylobacter* infections in man. However, there has been a recent report which suggested that after chicken, salad vegetables and bottled water could be the most significant source of *Campylobacter* infections (Evans, Riberio and Salmon, (2003)¹⁵). In addition, there is evidence that there is significant *Campylobacter* contamination of livestock at dairy and beef farms.
- 8.3.4 No progress has been made in identifying the patterns of infections in man and the causes of these patterns. The questions on this issue raised in the 1999 MSFFG *Campylobacter* report reflected the infrequency of identification of *Campylobacter* infections. There is no explanation of why this latter point is the case.
- 8.3.5 The study of the epidemiology of campylobacters through the research described in this report does not show any clear patterns in terms of specific serotypes or strains. This is likely to result from a combination of the absence of standardised sampling and detection methods (see Section 5 above), the generally reported genetic instability of the organism and the absence of any reported differences between pathogenic and non-pathogenic strains. There is also a lack of consistent approach between the various research groups undertaking epidemiological studies.
- 8.3.6 There is an absence of research aiming to link the genotypic information being obtained through the MLST classification scheme and gene function studies with the pathogenicity of campylobacters and their epidemiology.
- 8.3.7 There is also an absence of significant research to explore the host immune response to campylobacters.

¹⁵ Research funded by the PHLS, Wales.

9. SURVEILLANCE

- 9.1 It was recommended in the 1999 MSFFG *Campylobacter* report that surveillance should be considered by the Microbiological Food Surveillance Group (now disbanded), the Epidemiology of Foodborne Infections Group and the Veterinary Surveillance Group set up under the Chief Veterinary Officer. Therefore, projects on surveillance would continue to be listed in the MSFFG database, but the work would not be discussed by the Funders Group.
- 9.2 There are several projects covered within this report which include elements of surveillance, and which are generally addressed elsewhere in the report. However, a significant study of the presence of *Salmonella* and *Campylobacter* in chicken for retail sale in the UK (**FSA B18002**) found that the overall frequency of contamination of chicken was 50%. Within this overall figure there was significant variation regionally (contamination was higher in Scotland and Northern Ireland than in England and Wales) and in the presentation of the chicken meat (whole bird or pieces, fresh or frozen). The highest levels of contamination were found in whole fresh chickens.
- 9.3 It was also observed that UK-produced chicken had higher levels of *Campylobacter* than imported whole birds.

10. RISK ANALYSIS

- 10.1 Risk assessment and analysis is an established process in many aspects of life, including the food industry and the management of human health. It is an iterative process, which requires that there is a regular consideration of what are the risks associated with a particular activity or item. In the case of *Campylobacter* research, there is a need to explore the risks to human health of the various sources of *Campylobacter*, the different strains, human practice and behaviour as well as aspects of the food chain and food production processes.
- 10.2 **Overview of current research**
- 10.2.1 An underlying issue is that there is less awareness or understanding within the food production industry (and the general community) of *Campylobacter* than there is with *Salmonella*. This was supported by research which considered information from the research literature, the poultry industry and analysis of farm to fork processes for chicken products (**FSA B03005**). The project found that the lack of understanding of many aspects of *Campylobacter* led to higher contamination of raw poultry meat (as compared with *Salmonella*). It was therefore important to understand better how poultry become

contaminated with *Campylobacter*.

- 10.2.2 Specifically within the meat production industry, research found that testing in meat plants for campylobacters was rare (**FSA M01014**) and a further project found, through use of questionnaires, that members of the British Meat Manufacturer's Association were more concerned about *Escherichia coli*, *Salmonella* and *Staphylococcus aureus* than *Campylobacter* (**MAFF FS3206**).
- 10.2.3 A particular concern for humans is infection with antibiotic resistant *Campylobacter*. The risk of such an infection being acquired from the food chain, or more widely, is being examined (**FSA B10004**). This should lead to an understanding of the contribution of the food chain to the occurrence of fluoroquinolone resistant campylobacters in man.
- 10.2.4 FSA project **B03005** found that, although a Quantitative Risk Assessment (QRA) model could be developed for *Salmonella* in the food production chain, this was not achieved (yet) for *Campylobacter*. However, a separate project has developed a QRA model for *Campylobacter* (**Defra OZ0608**) which could be used to identify and evaluate possible *Campylobacter* infection control measures. An issue for this work, recognised in other projects (**OZ0602, OZ0605**), is the problem of identification and categorisation of *Campylobacter* strains. This difficulty is compounded by the different options for identifying and differentiating between the organisms.
- 10.2.5 Comparison of the handling, preparation and cooking of chicken in domestic and catering establishments found that the probability of transferring contaminants from raw food to cooked food was high in the domestic context. However, the most important factor affecting the transfer of bacteria to finished food was the degree to which raw food was contaminated initially (**FSA B01015**).
- 10.2.6 Mathematical modelling of the entire food chain in relation to *Campylobacter* is proposed in order to provide a set of tools and techniques for future research (**BBSRC 0455**).
- 10.2.7 Concern has been expressed that a route for entry of pathogens, including campylobacters, into the food chain may be from livestock slurries to the wider environment. Exploration of this has started and work is being done to identify and prioritise all possible routes for such transfer (**Defra WA0804**).
- 10.2.8 One accepted approach to risk assessment is the use of Hazard Analysis Critical Control Point (HACCP) principles. The applicability of this approach in relation to possible contamination of wild game (**FSA M01025**) and sheep meat (**FSA M01015**) with pathogens (including *Campylobacter*) is being investigated and to date appears practical. However, there are clearly issues with the adoption of appropriate

biosecurity methods in farms and solutions to these are being researched (**Defra OZ0144**). Separately, a general HACCP-type scheme has been developed for beef production (**FSA M01009**).

10.2.9 Finally, work on the contamination and depuration (cleaning) of shellfish has been extended to include preliminary work in relation to *C. jejuni* (**FSA B04008**). However, it is not clear whether shellfish represent a significant route for introduction of the pathogen to the food chain.

10.3 **Assessment and Identification of gaps**

Gaps identified in 1999 MSFFG *Campylobacter* report

10.3.1 In the 1999 MSFFG *Campylobacter* report it was recorded that quantitative risk assessment for *Campylobacter* in the food chain was hampered by the lack of definitive information on the pathways of infection and associated subtypes. However, despite these limitations, risk assessment could 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 need for development of a preliminary risk assessment for the exposure of humans to campylobacters in food
- The issue of the contribution made by cross-contamination in the kitchen to human exposure to campylobacters.

Assessment of progress and identification of current gaps in research

10.3.2 The development of risk assessments for *Campylobacter* is clearly important with respect to control of the organism and management of public health. Work covered in this report does address some aspects of this. However, there is still a lack of research into the contribution of cross-contamination in the kitchen as a cause of human *Campylobacter* infections.

10.3.3 There is a certain amount of overlap between different projects considering the development of risk assessments for campylobacters. There is an absence of co-ordination aimed at providing agreed methods for risk assessments. This is partly because risk assessment studies are being undertaken in many contexts, supported by a range of funding bodies and other organisations.

10.3.4 The research does show, however, that a major risk to humans is the contamination of raw chicken brought into food preparation. As commented above (eg paragraphs 8.2.12, 8.2.16) there is a lack of

assessment of the relative risks of different possible routes for acquisition of *Campylobacter* infections by man.

- 10.3.5 Risk assessment is iterative, in that it constantly needs to be repeated so that new information can be incorporated and experience taken into account. Therefore it is important to ensure that there is continued, as well as co-ordinated, research in this area.

11. REDUCTION AND ELIMINATION

- 11.1 Research on the reduction and elimination of *Campylobacter* addresses the management of *Campylobacter* at every stage or opportunity in the food chain, as well as preventive measures which could limit the susceptibility of given populations to the bacterium, such as vaccines. Currently most research is directed at reduction in chickens, as this is perceived as offering the greatest gain in relation to the objective of reducing the levels of the organism entering the food chain.

11.2 Overview of current research

Management of *Campylobacter* in chickens

- 11.2.1 The possibility that pre-colonisation of chickens with non-pathogenic *Campylobacter* strains could prevent infection with pathogenic strains, and therefore reduce the risk of introduction of the pathogen to the food chain, provides the basis for research into competitive exclusion. It was observed that this approach was possible, as there is some evidence that during colonisation campylobacters generate an environment which is inhospitable to subsequent challenges (**Defra OZ0603**). However, subsequent research by the same group has not made progress as the researchers were unable to distinguish between pathogenic and non-pathogenic strains (**Defra OZ0606**).
- 11.2.2 It has been possible to develop chicken lines which are resistant to certain diseases, and this research is being extended to identification of chickens and chicken genes that are associated with *Campylobacter* resistance (**BBSRC 0891, D16959**).
- 11.2.3 Recently funded research is aimed at investigating whether the quality of poultry husbandry affects colonisation by *Campylobacter* (**FSA B15001**).

Physical reduction in bacterial load

11.2.4 One route to reduction of *Campylobacter* and other pathogens in the food chain is to reduce the levels associated with chicken carcasses. Possible techniques for achieving this have been developed and are in trials with appropriate equipment manufacturers (**FSA M01019**).

11.3 Assessment and Identification of gaps

Gaps identified in 1999 MSFFG *Campylobacter* report

11.3.1 In the 1999 MSFFG *Campylobacter* report it was observed that there was no reliable and consistent means of reducing *Campylobacter* infection in primary animal production and there was a lack of a reduction strategy in the slaughter-house and beyond. The following gaps in knowledge at the time were identified:

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

Assessment of progress and identification of current gaps in research

11.3.2 Understanding of the causes of *Campylobacter* contamination of chickens has increased as a result of several research projects. However, there are still significant issues to be addressed. For example, it is still not clear what are the sources of infection in poultry houses, although there is Defra funded research which aims to address this (Defra OZ0608).

11.3.3 Although there is limited research covered in this report on the reduction of *Campylobacter* infections at the farm, the issues have been considered and addressed by the ACMSF *Campylobacter* Working Group (ACMSF Annual Report, 2002) and the FSA document The Strategy for the Reduction of *Campylobacter* in Broiler Flocks (2003).

11.3.4 No work addressing the role or effect of vaccines has been undertaken within the research portfolio covered by this report.

12. MICROBIAL ANTIBIOTIC RESISTANCE

12.1 Resistance of bacteria to antibacterial compounds is an increasingly important issue, bringing with it long term implications for food safety and public health. Many factors contribute to the development of antibiotic resistance. These include medical and farming practices, the nature of the interaction between the antibacterial drug and the target bacterium and the genetic adaptability of the bacterium.

12.2 Research into the resistance of *Campylobacters* to antibiotics includes the routes by which the bacteria acquire antibiotic resistance, the mechanisms of resistance, the patterns of occurrence of resistant strains and the implications for human health.

12.3 Overview of current research

12.3.1 The antibiotics of choice used in the treatment of *Campylobacter* infections in man are erythromycin and fluoroquinolones such as ciprofloxacin. These are also used in the prevention and treatment of *Campylobacter* infections in poultry and pigs. There is concern relating to the increasing occurrence of antibiotic resistant strains of *Campylobacter*, including the possibility that the use of antibiotics in farming practice may lead to the introduction of antibiotic resistant strains to the food chain.

12.3.2 Determination of the antibiotic resistance of a large number of *Campylobacter* isolates revived from storage revealed that the majority of these strains were sensitive to ciprofloxacin (the antibiotic of choice for treating severe disease) and erythromycin, although resistance to the former was rising. Significantly, 26% of strains of the closely related *C. coli* were found to be resistant to ciprofloxacin (**FSA B10002**). In contrast, only 1.6% of over 400 isolates of *C. jejuni* were found to be resistant to ciprofloxacin, with higher numbers being resistant to erythromycin (18.7%) and ampicillin (6.8%).

12.3.3 Much of the work investigating antibiotic resistance in campylobacters has focussed on fluoroquinolones. A method has been developed to measure the concentration of ciprofloxacin and enrofloxacin in samples from chicken gut, serum and faeces. This will enable the estimation of concentrations of the antimicrobial compounds *in vivo*. (**Defra OZ0502**).

12.3.4 Several studies have shown that fluoroquinolone resistance is readily developed in *Campylobacter* (**Defra VM02100, OD2004, VM02105**). This occurred both *in vivo* (**VM02100, OZ0502**) and *in vitro* (**VM02100, OD2004**). It was also found that passage of *Campylobacter* with antibiotics or disinfectants gave rise to multiple antibiotic resistant strains of the pathogen (OD2004) but that, in contrast with *Salmonella*,

the use of both approaches, one immediately after the other, was not a double selection pressure for such strains. There was also no apparent link between resistance of *Campylobacter* to organic solvents and low level resistance to antibiotics (again in contrast with *Salmonella*) (**VM02100**).

- 12.3.5 The link between fluoroquinolone resistance and resistance to other antibiotics in campylobacters isolated from poultry was supported by research in **Defra OZ0501**. It was also found in this research that some of the resistant strains identified in poultry were also common in human infections.
- 12.3.6 The genetic basis of the antibiotic resistance (and sensitivity) of *Campylobacter* has been examined (**OD2004, VM02100**). It has been previously established that mutations in *gyrA* (DNA gyrase) were significant. New work investigated whether there was up-regulation of the expression of efflux pump genes such as *cmeB* (**VM02100, OD2004**). However, not all such genes, nor porins, showed changes in expression associated with antibiotic resistance (**VM02100**). It appears (**Defra OZ0501**) that the mechanism of resistance to antibiotics in campylobacters isolated from man and poultry is the same.
- 12.3.7 Although most of the work on antibiotic resistance of *Campylobacter* has focussed on fluoroquinolones, one project has looked at erythromycin. It is thought that resistance of campylobacters in pigs to erythromycin is the result of therapeutic use of erythromycin. However, investigation of the effect of treatment of chickens with the antibiotic did not lead to selection of resistant *Campylobacter* strains (**Defra OZ0502**).
- 12.3.8 If the use of fluoroquinolones is necessary in poultry management (**OZ0501**), and its use is associated with the development of antibiotic resistant strains, then it is helpful to know whether such strains exist in the absence of the use of the antibiotic, and after it has been withdrawn. It was found that resistant *Campylobacter* could be isolated from chickens and their environment in the absence of recent fluoroquinolone exposure. In addition, there were resistant strains persisting for up to four weeks after the withdrawal of the antibiotics (**OZ0501**).
- 12.3.9 With respect to the introduction of antibiotic resistant *Campylobacter* into the food chain, **FSA B18002** reports that 50% of *C. jejuni* isolates from retail chicken samples were resistant to at least one antibacterial drug (over 4800 samples were tested, of which half were found to be contaminated with *Campylobacter*). It was found that frozen chicken was significantly more likely to be contaminated with resistant *Campylobacter* than fresh chicken (59% as compared with 46%).
- 12.3.10 The effect of the use of quinolones (such as ciprofloxacin) in the food chain on quinolone resistance of *Campylobacter* is being

investigated (**FSA B10004**). Early information from the project has identified many different sources of potential infection of individuals by resistant *Campylobacter*.

12.4 Assessment and identification of gaps

Gaps identified in 1999 MSFFG *Campylobacter* report

12.4.1 The 1999 MSFFG *Campylobacter* report highlighted the observations of a report from the ACMSF (Report on microbial antibiotic resistance in relation to food safety, (1999)). In considering the complex issue of microbial antibiotic resistance in relation to food safety it regarded two areas as being of particular importance:

- Work was needed on the chain of events which could lead to antibiotic-resistant micro-organisms arising from farming practices being transmitted through food chain pathways and causing human infection
- Research was 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.

12.4.2 In the 1999 MSFFG *Campylobacter* report it was observed that at that time there had been very little research in this area with respect to *Campylobacter*, but considered it possible that in the light of the ACMSF report there could be an increase in activity.

Assessment of progress and identification of current gaps in research

12.4.3 The research supported by the MSFFG members in this area has focussed primarily on the emergence of resistant strains of *Campylobacter* in poultry (and to a lesser extent pigs). This research has not significantly addressed the issues of the persistence of resistance, or the epidemiology of resistant strains.

12.4.4 There are still limited amounts of data addressing the issue of the transmission of resistance through the food chain to man. There does not appear to be any information on the development of a reservoir of resistant campylobacters which could be transferred to man.

12.4.5 It may be that the lack of activity in this area reflects the nature of the clinical problem for *Campylobacter* infections. In general, *Campylobacter* infections go unmedicated, and antibiotics to control an infection are only introduced in severe cases (Defra OZ0501, COMIC (2000)). However, the incidence of ciprofloxacin-resistant campylobacters is rising and there is no body of research addressing

the cause and importance of this.

- 12.4.6 Equally, with the primary issue of *Campylobacter* infections in man being related to the contamination of uncooked food, it may be that research on antibiotic resistance in campylobacters is of a lower priority, other than as part of the general increase in understanding of the organism.

13. OTHER RESEARCH

- 13.1 In the 1999 MSFFG *Campylobacter* report, a particular socio-economic issue was identified where further research would be beneficial. This was the possible socio-economic costs of sequelae associated with *Campylobacter* infection, in particular Guillain-Barré syndrome. There are no projects in the current review of the MSFFG database which address this, or any other socio-economic subject.

14. RESEARCH INITIATED SINCE MID-2002

- 14.1 A number of projects referenced above have been initiated since mid-2002, and therefore have not led to significant findings so far. Broadly, these are in the areas of microbial physiology (**BBSRC D19661, D18084/18368, D18189, D19662, D15819**), fundamental genomics (**BBSRC EGA16166/67/74**), epidemiology (**FSA S01017, FSA B14012**), pathogenicity (**BBSRC 0941, D16959, FSA B15001**), antimicrobial resistance (**FSA B10004, Defra OD2010**) and risk factors (**FSA M01025, B14011, Defra OZ0144**).

15. CONCLUSIONS

- 15.1 The research described in this report provides a view of the current understanding of many aspects of *Campylobacter*. Significant progress has been made in the last four years in the major area of classification of the many variants of the organism, and also in fundamental understanding of the organism. It is now important that a common genomic classification system is agreed between the funding bodies and research community. Current thinking would suggest that MLST should be core to this scheme.
- 15.2 There is less progress to report in the understanding of the epidemiology and pathogenicity of the organism. This is partly a result of the absence of an agreed classification system which can be readily and consistently applied by all research groups.
- 15.3 The presence of several funding bodies with similar interests in *Campylobacter* research has led to a number of similar projects being funded without a mechanism for bringing the data together, or for undertaking meta-analysis of the results. Areas where this has been most apparent are sample preparation methodologies and classification schemes. However, with increasing interaction among members of the *Campylobacter* research community (for example the CampyUK meetings) as well as among the funders, management of these issues is being addressed.
- 15.4 The extensive body of research on *C. jejuni* molecular biology, genetics and physiology provides an excellent resource for other research groups within the UK. There are clearly opportunities for synergy, for example using the microarrays developed through **BBSRC BFP11362** to develop rapid identification of *Campylobacter* species (**FSA B14012**). There are other opportunities, such as the use of gene knock-out mutants (**BBSRC EGA16166/67/74**) and STM (**Defra VF 0101**) for identifying and understanding important properties of the organism in relation to virulence, colonisation and host-pathogen interactions.
- 15.5 It is clear that there is relevant research on *C. jejuni* and campylobacters in general which is not funded by the members of the MSFFG, in particular outside of the UK. There would be benefit in developing an awareness of this research in order to understand the wider picture of progress with *Campylobacter* research. It could be that such an approach would enable further clarity in relation to overlaps and gaps in the research. This could be done initially at a national level, but would eventually need to consider international funding of work on *Campylobacter*.

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APPENDIX 1: GLOSSARY

AFLP

Amplified Fragment Length Polymorphisms. Molecular markers typical of a strain of an organism obtained by combining RFLP and PCR techniques and applying these to restriction fragments obtained from a total genomic DNA digest of an organism.

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*.

CDT

Cytolethal distending toxin, produced by *C. jejuni*. Implicated in *C. jejuni* pathogenicity in man, but not proven to be essential.

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

See TCA cycle below

Culturable/non-culturable

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

Gluconeogenesis

The metabolic formation of glucose from non-carbohydrate precursors. The enzymes of the pathway are in many cases the same as those involved in the corresponding reverse step in the glycolytic pathway.

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.

Lightcycler™

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

Lipo-oligosaccharide (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.

Lipo-polysaccharide (LPS)

The endotoxic component of the outer membrane in Gram negative bacteria. LPS is antigenically variable and 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.

MLST

Multi locus sequence typing (MLST) is an unambiguous procedure for characterising isolates of bacterial species using the sequences of internal fragments of seven house-keeping genes.

Palindromic DNA sequence

A double-stranded DNA sequence which reads the same from left to right as from right to left.

PCR

Polymerase Chain Reaction. Method for amplifying a specific sequence of nucleotides within double-stranded DNA.

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 white blood cells, particularly neutrophils and macrophages, which are able to ingest and then digest particulate matter.

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 arises from the insertion or deletion of DNA and can be used to identify different genotypes.

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.

Restriction enzymes

Enzymes which recognise short palindromic sequences in DNA and cut the double helix at a particular point in the sequence. There are many such enzymes, recognising different DNA sequences.

Ribotyping

Method of determining the relatedness of a group strains of an organism by comparing the pattern of DNA fragments obtained after cutting of the ribosomal RNA genes with selected restriction enzymes.

Socio-economic burden

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

STM

Signature tagged mutagenesis is a means of identifying virulence genes in bacteria which are essential for the process of infection in a chosen animal model.

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.

TCA Cycle

Tricarboxylic Acid cycle (citric acid cycle). A cyclic sequence of reactions that plays a central role in the metabolism of many microorganisms and involves several tricarboxylic acid intermediates.

Viable

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

VNC

Viable, non-culturable bacterial cells which are alive but not capable of growth in culture medium.

APPENDIX 2: PROJECTS FROM THE MSFFG DATABASE USED IN THIS REPORT

Projects are sorted by funder and then by project code.

Project Code	Project Title	Funder id	Start Date	End Date	Cost
0455	Systems analysis for risk assessment	BBSRC	01-Apr-2001	31-Mar-2004	
0891	Molecular genetics of the host response to zoonotic infections in chickens	BBSRC	01-Oct-2001	30-Sep-2004	
0941	<i>Campylobacter jejuni</i> infection in chickens - intestinal colonisation and host pathogen interactions	BBSRC	01-Jun-2002	31-May-2005	
3258371	Microbial adaptation to environment and survival in the food chain	BBSRC	01-Apr-1998	31-Mar-2001	£640,232
4301055A	Bacterial adaptation & evolution in response to environmental stress	BBSRC	01-Apr-1999	31-Mar-2000	£264,350
4311209	Molecular genetics of <i>Campylobacter</i>	BBSRC	01-Apr-2000	31-Mar-2003	
BFP11294	An analysis of carbon metabolism and alternative respiratory pathways in <i>Campylobacter jejuni</i>	BBSRC	01-Oct-1999	01-Oct-2002	£207,652
BFP11346	Respiration and oxygen tolerance in <i>Campylobacter coli</i> and <i>C. jejuni</i> : implications for food safety and colonisation	BBSRC	01-Jul-1999	13-Nov-2002	£373,968
BFP11362	Construction and application of a <i>Campylobacter jejuni</i> DNA microarray to investigate differential gene expression	BBSRC	01-Jul-1999	01-Jul-2002	£196,864
BFP11390	Post-genomic analysis of the proteome of <i>Campylobacter jejuni</i> (joint with BFP11391/92)	BBSRC	01-Jul-1999	01-Jul-2002	£178,928
BFP11391	Post-genomic analysis of the proteome of <i>Campylobacter jejuni</i> (joint with BFP11390/92)	BBSRC	01-Jul-1999	01-Jan-2002	£119,316
BFP11392	Post-genomic analysis of the proteome of <i>Campylobacter jejuni</i> (joint with BFP11390/91)	BBSRC	01-May-1999	01-May-2002	£192,684
D09207	The <i>Campylobacter jejuni</i> RacR regulatory system : characterising the regulon and its role in adaptive responses and intestinal colonisation	BBSRC	28-Feb-1998	28-Feb-2001	£162,169

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Project Code	Project Title	Funder id	Start Date	End Date	Cost
D13925	Development of a universal DNA microarray to investigate genome diversity in <i>Campylobacter jejuni</i>	BBSRC	01-Oct-2000	01-Oct-2003	£210,856
D14376	Diarrhoeal mechanisms of <i>Campylobacter jejuni</i>	BBSRC	18-Jun-2001	18-Jun-2004	£176,320
D14520	The role of anaerobic respiration in survival, colonisation and pathogenicity of <i>Campylobacter jejuni</i>	BBSRC	01-Oct-2001	01-Oct-2004	£181,216
D15819	An investigation into multiple glycosylation in <i>Campylobacter jejuni</i>	BBSRC	24-Jul-2002	24-Jul-2005	£204,012
D16959	An investigation into host genetic resistance to campylobacter colonisation in poultry	BBSRC	27-May-2002	27-May-2005	£254,768
D18084	Mechanisms underlying survival of <i>Campylobacter jejuni</i> during oxidative and nitrosative stresses (joint with D18368)	BBSRC	01-Aug-2003	01-Aug-2006	£220,788
D18368	Mechanisms underlying survival of <i>Campylobacter jejuni</i> during oxidative and nitrosative stresses (joint with D18084)	BBSRC	13-Nov-2002	13-Nov-2005	£185,256
D18189	Roles of electron transport through nitrate and nitrite reductases in survival, growth and colonisation of <i>Campylobacter jejuni</i>	BBSRC	01-Apr-2003	01-Apr-2006	£214,968
D19661	Iron responsive regulatory networks in <i>Campylobacter jejuni</i>	BBSRC	25-Oct-2003	25-Oct-2006	£158,548
D19662	Iron Responsive regulatory networks in <i>Campylobacter jejuni</i>	BBSRC	01-May-2003	01-May-2006	£152,088
EGA16166	Exploiting <i>Campylobacter jejuni</i> genomics	BBSRC	01-Aug-2002	01-Aug-2005	£314,776
EGA16167	Exploiting <i>Campylobacter jejuni</i> genomics	BBSRC	15-Feb-2003	15-Feb-2006	£380,844
EGA16174	Exploiting <i>Campylobacter jejuni</i> genomics	BBSRC	01-Sep-2003	01-Sep-2007	£77,676
9723	Microbial quality of beef carcasses in Northern Ireland abattoirs - A baseline study	DANI (DARD)	01-Apr-1997	31-Mar-2000	
9641	Molecular typing of <i>Campylobacter</i> species	DANI (DARD)	01-Jan-1997	01-Jan-2000	£56,200
0117	A comparison of poultry meat <i>Campylobacter</i> spp with those isolated from human disease	DARD	2002	2005	
R7105FOS	Comparative study of thermophilic <i>Campylobacter</i> isolates of clinical and food origin using genotypic and anti-microbial characterisation techniques	DARD	2002	2004	
ST200	Epidemiology, pathogenesis and control of campylobacter in commercial broiler flocks	DARD, InvestNI	01-Sept-2000	31-Aug-2004	

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Project Code	Project Title	Funder id	Start Date	End Date	Cost
FS3206	Efficacy testing of disinfectants used in the food industry against a range of pathogens including <i>E. coli</i> 0157	Defra	01-May-1998	01-Nov-1999	£129,923
OD2004	Loss of antibiotic resistance: analysis of phenotype and related gene expression	Defra	01-Apr-2000	31-Mar-2003	£240,869
OD2010	Use and abuse of non-antibiotic antimicrobials as major contributors toward the development of antimicrobial resistance	Defra	01-Oct-2003	30-Sep-2006	£433,926
OZ0132	Antibiotic Resistance mechanisms in <i>Salmonella</i> and <i>Campylobacter</i>	Defra	01-Apr-1997	31-Mar-2000	£231,613
OZ0144	Constraints to uptake of adequate biosecurity on UK cattle and sheep farms, with special reference to zoonotic diseases.	Defra	01-Jun-2002	30-Nov-2003	£113,270
OZ0501	Antibiotic treatment of commercial broiler flocks: incidence and mechanisms of fluoroquinolone resistance in <i>Campylobacter</i> .	Defra	01-Jan-2000	31-Mar-2003	£450,134
OZ0502	In vivo models to investigate the development of antibiotic resistance	Defra	01-Apr-2000	31-Mar-2002	£183,826
OZ0602	To Identify and Characterise campylobacters Virulent for Humans and Derived from Food Producing Animals.	Defra	01-Apr-1998	31-Mar-2001	£155,766
OZ0603	The Development of Non Pathogenic Strains of <i>Campylobacter jejuni</i> as Agents of Competitive Exclusion in Poultry.	Defra	01-Apr-1998	31-Mar-2001	£320,000
OZ0604	Characterisation of Strain Variation in <i>Campylobacter jejuni</i> .	Defra	01-Apr-1999	30-Sep-2004	£646,764
OZ0605	Genotypic and phenotypic instability of campylobacters from environmental, animal and human sources	Defra	01-Aug-1999	31-Jul-2002	£298,279
OZ0606	Protective immunity and competitive exclusion in development of effective intervention products for poultry	Defra	01-Apr-2001	31-Mar-2004	£232,145
OZ0607	An investigation of the distinguishing features of <i>C. jejuni</i> strains that have host/disease associations	Defra	01-Jul-2001	30-Jun-2004	£508,987
OZ0608	Epidemiological studies and development of practical control measures for <i>Campylobacter</i> in broiler flocks	Defra	01-Jul-2002	30-Jun-2006	£830,943
VF0101	Defra Cambridge Research Fellowship in Microbiology	Defra	01-Oct-1999	30-Sep-2004	£1,250,773
VF0201	Defra Liverpool Veterinary Research Fellowship in Epidemiology	Defra	01-Sep-1999	31-Aug-2004	£1,245,279
VM02100	Factors influencing the development of resistance to fluoroquinolone antibiotics by food borne bacteria	Defra	01-Apr-2000	30-Sep-2003	£366,348

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Project Code	Project Title	Funder id	Start Date	End Date	Cost
VM02101	Assessment of factors influencing the development of resistant to fluoroquinolone antibiotics used in pigs and poultry.	Defra	01-Jul-2000	30-Sep-2003	£490,863
VM02105	Identification and use of genomic markers of antibiotic resistance in campylobacters, salmonellae and enterococci.	Defra	01-Aug-2000	31-Jul-2003	£279,289
WA0656	implications of potential measures to control pathogens associated with livestock manure management	Defra	01-Nov-2000	30-Oct-2001	£65,050
WA0804	Routes by which pathogens associated with livestock slurries and manures may be transferred from farm to the wider environment	Defra	01-Sep-2001	30-Nov-2004	£298,090
B01005	Variations in the virulence of <i>Campylobacter jejuni</i> strains associated with poultry and poultry meat	FSA	01-Apr-1998	31-Mar-2001	£198,129
B01015	Determine exposure assessment & modelling risks associated with the preparation of poultry, catering & home	FSA	01-Jun-1999	30-Jun-2001	£125,695
B02013	A study to examine the contamination of catering and economy sausages with Salmonella spp.	FSA	01-Mar-2000	31-Oct-2000	£29,827
B02015	A national survey of potential cross-contamination resulting from kitchen cloths in domestic kitchen	FSA	01-Aug-2000	31-Mar-2002	£258,943
B02016	Microbiological risk factors associated with the domestic handling of meat	FSA	01-Nov-2000	31-Oct-2002	£158,605
B03001	Field studies to identify and evaluate key intervention points for salmonella control during Broiler production	FSA	01-Jul-1997	30-Jun-2000	£202,197
B03003	The molecular epidemiology of campylobacters in poultry and poultry meat and use to develop intervention strategies	FSA	01-Apr-1997	31-Mar-2000	£248,315
B03005	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	FSA	01-Sep-1998	31-Aug-1999	£110,662
B03008	Studies to identify critical points for infection of live birds or contamination of poultry carcasses with <i>Campylobacter</i> and <i>Salmonella</i>	FSA	01-Nov-1999	01-Feb-2003	£359,921
B03010	Efficacy of water disinfection systems for broiler production unit	FSA	01-Oct-1999	30-Jun-2002	£534,930
B03011	"Real time" PCR detection and speciation of <i>Campylobacter</i>	FSA	01-Apr-1999	31-May-2000	£53,268
B03013	Comparison of clinical <i>Campylobacter jejuni</i> in North West England and Northern Ireland	FSA	03-Dec-2001	31-Jan-2004	£32,296

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Project Code	Project Title	Funder id	Start Date	End Date	Cost
B03014	Whole genome analysis Fluorescent Amplified Fragment Length Polymorphism (FAFLP): Identification of post-specificity genes of <i>Campylobacter</i> and their values as epidemiological markers.	FSA	01-Jun-2000	30-Sep-2001	£69,081
B04008	Application and validation of techniques for the detection of pathogens in shellfish	FSA	01-Aug-2000	30-Nov-2002	£204,150
B05003	Pathogens in organic wastes: their levels and survival both during storage and following application to agricultural land	FSA	01-Jul-1999	01-Feb-2003	£837,860
B05008	The levels of pathogens in abattoir wastes	FSA	01-Nov-1999	28-Feb-2002	£210,067
B08008	Methods used for the assessment of the number and prevalence of Salmonella and <i>Campylobacter</i> spp. in chicken on retail sale	FSA	01-Nov-1999	30-Jun-2000	£11,457
B09002	Rapid detection, quantification and molecular characterisation of thermophilic <i>Campylobacters</i> in foodstuffs and related environments	FSA	10-Jan-1996	31-Dec-1999	£110,157
B09005	Review of microbiological methods in the food industry	FSA	01-Jun-1998	31-Mar-2001	£115,579
B10002	Drug resistant screening of <i>Campylobacter</i> isolates from IID study	FSA	01-Jan-2000	31-Mar-2000	£10,491
B10004	Assessment of, relative to other pathways, the contribution made by the food chain to the problem of quinolone resistance in microorganisms causing human infections	FSA	01-Apr-2002	31-Mar-2005	£507,070
B14011	Case control study of risk factors for <i>Campylobacter jejuni</i> infectious intestinal disease in England and Wales	FSA	01-Apr-2003	30-Apr-2005	£309,064
B14012	Environmental and water-borne sources of <i>Campylobacter</i> in the North west of England and their influence on seasonal human infection	FSA	01-Apr-2003	31-Mar-2006	£339,762
B15001	<i>Campylobacter</i> spp in housed broiler flocks: the influence of flock health, performance, husbandry and vaccination against other diseases on susceptibility to colonisation with campylobacter spp	FSA	01-Jun-2003	31-May-2005	£211,721
B17002	Assessment of the risks to food safety associated with spreading of animal manure and abattoir waste on agricultural land	FSA	01-May-2001	30-Apr-2002	£48,753
B18002	UK-wide survey of <i>Salmonella</i> and <i>Campylobacter</i> in fresh and frozen chicken on retail sale	FSA	26-Mar-2001	29-Jun-2001	£237,000

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Project Code	Project Title	Funder id	Start Date	End Date	Cost
ZB00023	Review of testing and scheduling practices for Salmonella and Campylobacter undertaken by the UK poultry industry and to determine the factors affecting their use	FSA	02-Jan-2002	02-Jul-2002	£43,500
ZB00030	Current Status Of, And Future Developments For <i>Campylobacter</i> Biosecurity At The Broiler Farm	FSA	02-Jan-2002	30-Apr-2002	£44,979
ZB00033	Poultry transport crate hygiene	FSA -	02-Jan-2002	02-Jul-2002	£40,634
M01009	Source and spread of particulate and bacterial contamination between cattle during the farm-to-abattoir phase of the production cycle	FSA	01-Oct-1999	30-Sep-2003	£284,190
M01011	A Study of Factors affecting MHS scores of sheep surviving at abattoirs and bacterial contamination of their carcasses	FSA	01-Jan-2000	31-Dec-2000	£24,938
M01013	Farm management practices to improve the visible and microbiological cleanliness of cattle hides at slaughter	FSA	01-Jan-2000	30-Sep-2003	£324,771
M01014	Microbiological verification of HACCP in meat plants	FSA	01-Apr-2000	01-Apr-2001	£57,631
M01015	Factors affecting the presence and spread of human bacterial pathogens in sheep	FSA	01-Jan-2000	31-Dec-2003	£396,856
M01017	Standardisation of sampling and analysis in poultry abattoirs in support of HACCP-based hygiene solutions.	FSA	01-Oct-2001	30-Sep-2004	£254,212
M01019	Physical methods readily adapted to existing commercial lines for reducing pathogens, particularly campylobacters, on raw poultry	FSA	01-Oct-2001	31-Mar-2004	£209,556
M01025	An evaluation of the effect of EU proposals to inspect licensed premises on the marketing of wild game: a qualitative risk assessment.	FSA	01-Dec-2002	30-Apr-2003	£29,837
S01005	National Food Surveillance System For Scotland	FSA - Scotland	01-Mar-2001	31-Mar-2004	£402,500
S01017	Detailed analysis of data arising from the Scottish component of a UK-wide study to describe human campylobacter infection	FSA - Scotland	01-Dec-2002	03-Apr-2003	£12,000
URG/001/96	Solid phase rapid detection of viable micro-organisms using nucleic acid amplification and biosensing techniques	SEERAD	01-Apr-1996	30-Sep-1999	£310,000