

FOOD STANDARDS AGENCY

B14 FOODBORNE DISEASES RESEARCH PROGRAMME REVIEW PROCEEDINGS

**Held at the
Training & Conference Centre, Radcliffe
House, Warwick University, Coventry, UK**

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1. EXECUTIVE SUMMARY

Over seventy delegates¹ from different sectors of industry, academia and Government attended the review of the Food Standards Agency (FSA) Research Programme B14 on Foodborne Diseases.

The main aims of the review were:

- To facilitate and conclude independent expert evaluation of individual projects and the FSA's overall programme on foodborne diseases, especially programme B14, and
- To inform stakeholders of the results of the programme funded by the FSA to date; and to seek ideas and input to inform future developments in this area of scientific research and FSA policy.

The review also evaluated how well the research had met the aims of the programme's original ROAME², and the rationale for the programme. In order for this evaluation to be carried out the contractors for the research projects were invited to present their results to an external panel of independent experts in open session, chaired by Professor Bill Reilly.

The expert panel also met in private session to evaluate each research project for scientific quality, relevance to FSA policy and value for money. The research contractors have been informed of the panel's evaluation.

¹ A list of attendees is included under appendix 2

² The B14 ROAME document is available at appendix 4

2. SUMMARY OF RECOMMENDATIONS

The following summarises the key recommendations made by the delegates and expert panel during the three-day event:

General recommendations for the FSA

Delegates highlighted that they felt the Agency was focussing on too many targets and there was a tendency to look for policy driven outcomes, even when the science available was not yet ready to support this. It was suggested that the Agency should adopt a more strategic approach which concentrates on fewer targets and investigating them in more depth. It was emphasised that it was important to ensure that adequate basic science research is carried out before commissioning applied work. Closer collaboration between the FSA, Defra and BBSRC to address the gap between the fundamental and more applied research was suggested as a way of achieving this.

It was suggested that it should be recognised that there are some nationally important strategic objectives that need to be addressed for the health of the nation that require a greater level of co-ordination and co-operation from the main funding bodies. Climate change and its impact on foodborne (and other) disease was cited as an example. The Panel were aware that the Agency is joint funding some recent research projects with the BBSRC through the Government-Partnership Award Scheme and fully endorsed this mechanism of funding.

There was a lack of understanding by both the Panel and the delegates at the review as to how the FSA defines its research priorities. Improving understanding of these processes by the wider research community was felt to be important.

It was noted that much research in the Foodborne Diseases programme was carried out by a small number of contractors and it was suggested that this may be a wider Agency issue. Some members of the Panel suggested that Agency funding was not seen as “first tier” (as opposed to BBSRC, MRC etc), particularly by Universities and hence research calls may not always attract the best calibre research scientists. A possible explanation was partly that the wordings of research requirements were too specific, which subsequently led to restrictions on the type of proposals being submitted in response.

Overall recommendations for the programme

The Panel and delegates suggested that the Agency future priorities in foodborne disease should focus more heavily on *Campylobacter* and recommended that the Agency should produce a new, more flexible research programme with the specific target of identifying steps to help reduce levels of *Campylobacter* infection. The other key theme that emerged was that research should focus more on the host with regard to the host/pathogen interaction, particularly with pathogens such as *Campylobacter* and *Listeria monocytogenes*. Investigating host susceptibility and behaviour (which should involve social science) was felt to be particularly important.

The main research priority areas identified by the B14 review delegates were:

- Cost effective studies of foodborne disease taking into account acute infection and chronic sequelae

- Host susceptibility and the behaviour of pathogens such as *Campylobacter* and *L. monocytogenes*
- Understanding the basic biology of *Campylobacter*
- Development of more refined tools for detecting *Campylobacter*
- The need for a 'PulseNet' equivalent in the UK for all foodborne pathogens
- Evolution of Clostridia strains and the lateral transfer of toxigenic genes
- More fundamental work on the distribution of heat resistant and sensitive spores in the environment
- Physiology and behaviour of foodborne pathogens

3. FSA ACTION PLAN

1. To inform contractors of the outcome of the independent evaluation of the B14 research projects.
2. To publish the B14 Foodborne Diseases Research Programme Review proceedings on the FSA website.
3. To consider and prioritise internally and with key stakeholders the recommendations of the review in support of an ongoing strategy on the Foodborne Diseases Research Programme.
4. To consider whether there is scope and sufficient justification for a continued programme on Foodborne Diseases research.

4. SUMMARY OF PRESENTATIONS

SESSION I: INTRODUCTION

The Chairman, Professor Bill Reilly (Food Standards Agency Board Member), welcomed delegates to the Review of the Food Standards Agency's Foodborne Diseases Research Programme and introduced the members of the Expert Panel³:

Professor Tom Humphrey
University of Bristol

Professor Christine Dodd
University of Nottingham

Professor Mike Gasson
Institute of Food Research

Professor Charles Penn
University of Birmingham

Dr John Cowden
Health Protection Scotland

Dr Dominic Mellor
University of Glasgow

Professor Reilly informed the audience that Dr John Cowden was unable to attend the review open sessions but would be dialling into the review's closed session. He then invited Ms Gael O'Neill (Food Standards Agency, Microbiological Safety Division) to give an overview of the Foodborne Diseases Research Programme and the review process.

Background to the Food Standards Agency's B14 Foodborne Diseases Research Programme

Ms Gael O'Neill, Food Standards Agency's Microbiological Safety Division

The Food Standards Agency's research portfolio on food safety is divided into a number of research programmes, each of which has specific scientific aims linked to the Agency's strategic plan. The Agency's research portfolio was reviewed in May 2000 and 'A review of the Food Standards Agency's Research Portfolio and Research Management Systems'⁴ (the Arbutnott Review) was published in July 2001. The review recommended that each of the Agency's existing research programmes should be reviewed according to a clearly defined timetable to ensure they met the aims and objectives of the Agency, were providing value for money and if necessary, to direct future research.

³ Brief description of the B14 Foodborne Disease Chair and Expert Panel Member's background and area of expertise has been provided under Appendix 3

⁴ Food Standards Agency (2001). A Review of the Food Standards Agency's Research Portfolio and Research Management Systems. Published by the Food Standards Agency. ISBN 1 904026044 FSA/0046/0601

In response to this recommendation, the Food Standards Agency has undertaken to review all its research programmes by means of a formal independent evaluation. The review is an opportunity for the Agency to 'take stock' of completed research and identify areas that still need addressing. The Food Standards Agency's Microbiological Safety Division has reviewed 8 of its research programmes⁵. These include:

- B15 poultry research programme (13 January 2004)
- B16 shellfish hygiene research programme (27 and 28 January 2004)
- B15 eggs research programme (29 June 2004)
- B17 organic waste to land research programme (1 October 2004)
- B11 verocytotoxin producing *E. coli* (VTEC) research programme (18 January 2005)
- B20 domestic sector hygiene and B13 microbiological risk management research programme (29 and 30 June 2006)
- B12 microbiological risk assessment research programme (21 and 22 March 2007)

The main aim of this review was to evaluate each of the research projects funded under the B14 Foodborne Diseases programme and determine how well the research had met the aims and objectives of the B14 programme's original ROAME (Statement of each programme's **R**ational, **O**bjective, **A**ppraisal, **M**onitoring and **E**valuation). The review also provided a mechanism for informing stakeholders about the results of the Foodborne Diseases research funded by the Agency to date.

In 2000, the Food Standards Agency set itself a target to reduce the incidence of foodborne disease by 20% over a 5 year period. The foodborne disease strategy identified 5 key foodborne pathogens (*Salmonella*, *Campylobacter*, *Listeria monocytogenes*, *E. coli* O157 and *Clostridium perfringens*) against which the target to reduce laboratory reports was measured. In 2000 there were 65,643 laboratory reports for these 5 pathogens and this established a base-line figure against which the FSA target would be measured. In 2005 there were 53,052 reports which gave a reduction of 19.2%. The FSA's strategic plan for 2005 to 2010 is to reduce foodborne disease further. In order to reduce food poisoning, research needs to be funded to improve our understanding of the physiology, virulence and epidemiology of the 5 main food poisoning organisms and to develop suitable typing methods to achieve this.

The B14 foodborne diseases programme was designed to improve the understanding of the behaviour and epidemiology of foodborne organisms. The research programme involved investigating options for improving surveillance of gastroenteritis in humans (B08006, B14001, B14004, B14005 and B18021) as well as targeting specific causes of food poisoning. These include *Campylobacter* and *Salmonella* (B01013, B03013, B03014, B14011 and B14012), *Clostridium perfringens* (B14006, B14008 and B14009) and other less known Clostridia (B14007) and the detection/typing methods for Enteroaggregative *E. coli* (B14002 and B14003). The B14 programme specifically aimed to:

- Assess the contribution made by the food chain to the problem of sporadic cases of *Campylobacter* infection in humans, relative to other pathways

⁵ Further information on the Food Standards Agency's Microbiological Safety Division's research programmes is available at: <http://www.food.gov.uk/science/research/researchinfo/foodborneillness>

- Determine the contribution made by foreign travel to the overall burden of food poisoning
- Compare the incidence of *Campylobacter* in different parts of the UK
- Determine the physiology of lesser known microorganisms that may contaminate food
- Determine best practice for cooling large bulk meats to reduce outbreaks due to *C. perfringens*
- Investigate options for improving surveillance of gastroenteritis in humans
- Evaluate and standardise epidemiological tools for the study of foodborne disease

The programme aimed to provide a better understanding of the main food poisoning organisms and a microbiological framework for the development of intervention strategies to reduce foodborne disease.

The B14 programme review was arranged into 6 sessions:

- Session I: Introduction
- Session II: Review of the FSA's B14 epidemiology of foodborne disease research
- Session III: Review of the FSA's B14 *Campylobacter* and *Salmonella* research
- Session IV: Review of the FSA's B14 Enteroaggregative *E. coli* research
- Session V: Review of the FSA's B14 Clostridia research
- Session VI: Open discussion including future research priorities

A private expert panel meeting was held at the end of each day where the independent panel members evaluated and scored the projects for scientific quality and policy relevance.

Ultimately the review will enable the FSA to provide more accurate information based on relevant and up to date research. It will help the FSA provide guidance and advice to different sectors of the food chain, including farmers, food processors and manufacturers as well as allowing future advice to consumers to be more accurate and effective. It is expected that the review would also provide the basis for further discussion and assist in the identification of current gaps in knowledge and help to ensure that future research is appropriate whilst also reducing duplication of research effort.

Main discussions points

Clifton Gay (Food Standards Agency) asked Gael O'Neill to define the term ascertainment. Gael clarified that it is the process of discovering the number of cases of a disease that have occurred. One of the difficulties with foodborne disease is that there is no actual accurate measure of it and it is widely known that gastrointestinal infectious disease is vastly underreported. The first IID study estimated that for every single case that was included into the National Statistics, there was a further 136 cases in the community. So part of the B14 research programme was assessing approaches that could be used to calibrate the National Surveillance System and use the data more effectively.

Charles Penn (University of Birmingham) suggested that the 19.2% reduction in cases of the 5 measured pathogens corresponded to a period of time where there were a number of changes in both the NHS and the HPA. This is an important aspect of ascertainment, as there is a need to determine whether changes in practice had an

effect on the number of cases being confirmed in laboratories. Gael confirmed that a 19.2% decrease in laboratory reports was seen but that it was difficult to know whether the changes in the system meant that the laboratory reports numbers changed without any actual change occurring to rate of foodborne diseases. One of the main reasons for commissioning Project B18021: The Second Study of Infectious Diseases in the UK (the IID2 study) was so that the current data could be compared with the results from the Study of infectious Intestinal Diseases in England (the IID study), conducted in the mid-1990s to see whether there had been changes in the reporting pyramid.

Diane Newell (Veterinary Laboratories Agency) commented that the FSA spends between £25-30 million on science and asked what proportion is spent on food safety. Gael recalled that in 2003/2004 a third of the FSA's total science budget was spent on food safety but was unable to provide more recent figures. Bill Reilly (Food Standards Agency Board Member) added that it was important to bear in mind that food safety is competing with other priority areas in the FSA, such as diet and nutrition, which have a higher profile than they might have had 5 years ago. Paul Cook (Food Standards Agency) also added that the FSA remains committed to supporting food safety along with other areas such as healthy eating.

SESSION II: REVIEW OF THE FSA'S EPIDEMIOLOGY OF FOODBORNE DISEASES RESEARCH

Project B08006: Ascertainment and enhancement of gastrointestinal infection surveillance and statistics (AEGISS)

Dr Peter Hawtin, Health Protection Agency, Southampton General Hospital

The contractor's B08006 project summary is included in Appendix 9.

The main aims of the project were to develop a spatio-temporal statistical modelling system for the display and analysis of epidemiological outputs and other information sources based on a geographical information system platform, and to develop a system for collating and managing disparate data sources from multiple microbiology laboratories in a defined area.

An extensive collaboration was developed between academia, primary care (including NHS Direct) and five microbiological laboratories. Novel spatio-temporal statistical analysis was developed and applied using an automated data interface and a 24 hour web-based reporting system was developed. The work established a risk surface of the study area based on analysis of data derived from routine NHS Direct calls for gastrointestinal symptoms. Results from routinely submitted faecal samples to the collaborating laboratories and selected data from calls for gastrointestinal symptoms from NHS Direct were received daily. Analysis of the statistical model and the microbiology output identified areas of statistical anomalies where the frequency of calls to NHS Direct exceeded expected levels with a high degree of probability. In one incident, the statistical model identified an area with significant increase over expected calls to NHS Direct. Some days following this event, there were microbiologically confirmed cases of *Cryptosporidium* from the same area.

Overall the project demonstrated that spatio-temporal statistical modelling of non-specific gastrointestinal cases could identify anomalous locations for further

investigation to detect potential common source outbreaks. The project was successful in developing a system capable of capturing, collating, analysing and displaying surveillance data from different and disparate sources in real time. Syndromic surveillance increases sensitivity and may identify associated cases in space and time where traditional microbiological investigation could fail to detect because of lack of sensitivity.

Main discussion points

Bill Reilly commented that the presentation highlighted the difficulties involved in harnessing the dataset into a useable tool.

Tom Humphrey (University of Bristol) was intrigued by the differences between GPs and GP practices, and asked Peter Hawtin to provide more information on the reasons (e.g. age, background, education) for these variations. Peter suggested that these differences were mainly due to the variety of practice between GPs. Some GPs claimed they had never made a statutory notification. Younger GPs did not perceive public health to be a priority issue whereas the older GPs did. The monetary reward for making statutory notification is not very high and perhaps not much of an incentive to the GPs.

Mike Gasson (Institute of Food Research) asked whether the nature of the causative agent affected the time lag seen in data reporting. Peter said that the utility of the AEGISS system was not affected by the nature of the cause or time lag, as it was possible to alert GPs and Consultants in Communicable Disease Controls (CCDCs) very early and improve the sampling frame. GPs could be kept interested by closing the feedback loop.

Meirion Evans (Cardiff University) asked whether the variation in the rate of positive samples coming from different GPs could be a result of some GPs being more able to judge which patients are likely to be suffering from foodborne disease. He questioned whether there was any indication of how the specimens tested were selected? Peter replied that there was not really any indication of how specimens were selected. This could perhaps range from gaining some thinking time while the specimen was examined to a real requirement for a definite diagnosis of infection.

Tom Humphrey asked whether the GPs paid for the testing to be carried out and if so did this put them off sending the samples for testing. Peter confirmed that the GPs paid for testing as part of routine block contract with the Trust, but the costs were small and was not prohibitive. There was no additional cost to GPs for purposes of the project.

Mike Carter (University of Surrey) enquired whether there was any potential for using the model to show the data as a movie. Peter confirmed that there is an interactive website that shows the data moving over time. However, there is more interest in point significance. The system failed to detect an outbreak of *Cryptosporidium* in the under 5s as mothers tend to go straight to a GP for consultation rather than using NHS Direct. Mike also asked whether the AEGISS models exceedence sensitivity could be reduced and whether the NHS Direct call densities matched the population density. Peter confirmed that the model is adjustable but it is uncertain if the call centres and population densities matched as many procedures have changed - there are less call centres and there have also been changes in gastrointestinal infection algorithm.

Dominic Mellor (University of Glasgow) asked Peter to comment on the ability of the AEGISS model to pick up outbreaks where the location of patients in an outbreak (i.e. their home addresses) was not the place of exposure. Peter clarified that travel can be excluded as the questionnaire used to identify risk factors included specific questions on travel history.

Will Waites (University of Nottingham) asked what proportion of samples sent for testing were confirmed as positive and did this vary over time? Peter confirmed that 5-10% samples were positive and that this remained consistent during the study.

Project B14001: Food poisoning and foreign travel - study of population health burden and risk factors for antibiotic resistance

Dr Meirion Evans, Cardiff University

The contractor's B14001 project summary is included in Appendix 11.

The main objectives of the study were to quantify the incidence in the general population of travel-related gastrointestinal illness using population health survey data, as well as establishing regional primary care sentinel surveillance for traveller's diarrhoea. In addition it was intended to identify risk factors for ciprofloxacin-resistant *Campylobacter* infection using a case-comparison study and also to compare clinical outcome of illness caused by ciprofloxacin resistant and sensitive *Campylobacter* infection also using a case-comparison study.

The findings of the study suggest a high burden of domestic and travel-related infection. As only a minority of patients consult a doctor and consulters differ from non-consulters, routine surveillance not only underestimates illness rates, but also gives a distorted impression of the epidemiology of the disease. Nevertheless sentinel primary case surveillance has the potential to monitor secular trends in traveller's diarrhoea and to help characterise population group or travel destinations associated with higher risk. Further, foreign travel remains the major risk factor for ciprofloxacin resistant *Campylobacter* infection and the source of infection for domestically acquired resistant infections appears to be largely the same as for sensitive infections. However, the role of bottled water as a source of both domestically acquired and resistant infections is unclear. Finally, ciprofloxacin resistant *Campylobacter* infection does not appear to carry any increased risk of adverse clinical consequences.

Main discussion points

With regards to the findings on bottled water, Christine Dodd (University of Nottingham) asked what advice should be given to travellers i.e. drink bottled or tap water. Meirion replied that it was very difficult to provide advice categorically and it would depend on the situation. For travel in the EU, tap water is treated and should therefore be safe to drink, whereas bottled water is less safe, so the advice would probably be to drink tap water. Whereas outside the EU and in more exotic destinations there is a balance of risk and bottled water would probably be more favourable. Christine asked Meirion to expand on the finding on sparkling water and whether there were any other risk factors associated other than the water itself. Meirion was unsure if the distinction between still and sparkling water was meaningful, as the study did not distinguish between spring and

mineral water. There is potential for further study to determine if this is a genuine risk factor or something that was not controlled for and for a microbiological study of different types of water.

Mike Gasson noted that the questionnaire included 2 country risk factors and 2 food risk factors. Were the food risk factors the same for different countries? Meirion explained that they were unable to look at this at such a detailed level due to the small number of samples for each country.

Dominic Mellor asked for further clarification on the approach of using case-case (Antibiotic resistant: Antibiotic sensitive ($Ab^R:Ab^S$)) methodology as opposed to case-control and whether this could be misleading in terms of risk factors identified. Meirion suggested that if the risk factors are the same for both Ab^R and Ab^S cases then the case-case approach will not identify these unless there is a considerable excess in exposure to the risk factor in one group compared to the other. The US FoodNet scheme case-control study had identified consumption of poultry in restaurants as a risk factor/pathway probably because it used healthy people rather than patients with Ab^S infection as controls. Dominic asked whether the case-case approach in this instance could produce results that could be misinterpreted, as they would relate to the risk of acquiring a resistant infection as compared to a sensitive one (i.e. given that a *Campylobacter* infection has been acquired) as opposed to relating to the risks of acquiring a resistant infection as compared to no infection at all (which is how most people interpret odds ratios derived from such studies). Meirion confirmed that a case-case study may miss some risk factors that are common to both groups but will not distort the factors detected.

Will Sopwith (Health Protection Agency) asked whether the odds ratios were adjusted during the risk analysis. Presumably there was a greater risk with more exotic travel and that it is more likely that symptoms will be reported after more exotic travel. Meirion agreed that patients were more likely to consult a GP after exotic travel, and in turn the GPs may be more likely to report patients presenting symptoms after exotic travel. This could not be excluded from the study although the numbers for travel outside Spain were relatively small, with Spain being the most popular destination. Will also asked if there is a standard algorithm for GPs (in Wales) to report travel destination when they request a faecal specimen test. Meirion confirmed that there was no such algorithm and that routine reporting depended on several factors including the information the GP put on a request form for samples, and what data the laboratory extracted from these forms. Therefore there may be distortion by exotic destinations.

Peter Hawtin asked whether the study looked at when ice is added to bottled water. Meirion indicated that this was not specifically investigated.

Peter Silley (MB Consult Ltd) asked whether the questionnaire asked if pets had been treated with antibiotics and if so what antibiotics were administered. Meirion confirmed that patients were asked whether they or their family were on antibiotics, but not their pets.

Diane Newell asked whether Meirion would reconsider the recommendation for drinking water in light of recent findings that drinking wine is protective against *Campylobacter* infection. Meirion claimed that there could be an association, for example, wine drinkers tend to drink less water but this was not asked during the study.

Project B14005: Apply molecular techniques to the nucleic acid archive generated from stool samples archived from the infectious diseases study including Project B14004: Generate an archive of extracted nucleic acid for IID archived faecal specimens

Dr Jim Gray and Dr Jim McLauchlin, Health Protection Agency

The contractor's B14004 and B14005 project summaries are included in appendices 14 and 15 respectively.

The aims of the project B14004 were to: generate an archive of DNA and cDNA samples from all the archived faecal samples from the IID study; assess the use of PCR and RT-PCR based procedures for detecting specific gastrointestinal pathogens in these DNA and cDNA samples; maintain the archive and assess the stability of DNA and cDNA for target detection during course of the study.

Generic DNA and cDNA extraction/generation procedures were established together with PCR assays for the detection of norovirus, rotavirus, sapovirus, *Salmonella enterica*, *Campylobacter* spp., enteroaggregative *Escherichia coli*, *Clostridium perfringens*, *Cryptosporidium* spp. and *Giardia* spp. A pilot study was conducted using a statistically significant sample of the previously identified 'positive' samples and $\geq 73\%$ of norovirus, rotavirus, sapovirus, *Salmonella enterica*, *Campylobacter* spp., enteroaggregative *Escherichia coli*, and *Cryptosporidium* spp. were re-detected. However the presence of only 68% of the *Giardia* spp and 34% of the *Clostridium perfringens* could be re-confirmed. A complete archive of cDNA and DNA for all 4677 samples was generated and maintained at 80°C. With the exception of giardial DNA, no statistically significant deterioration in the detection rate of viral cDNA, bacterial DNA and *Cryptosporidium* DNA was detected over 5 years of storage. Detection by PCR of giardial DNA was obtained from 80% of the extracts after 18 months and this reduced to 10-20% after 50 months.

The generation of the archive allowed the successful completion of project B14005 and proved the feasibility of using molecular tests in the IID2 project.

The main aim of project B14005 was to test all the archived DNA and cDNA extracted from the archived faecal samples from the IID study (FSA Project B14004) with PCR and RT-PCR based procedures for known and putative agents of infectious intestinal disease.

Project B14005 produced assays for the detection of norovirus, rotavirus, sapovirus, *Salmonella enterica*, *Campylobacter* spp., Enteroaggregative *Escherichia coli*, *Cryptosporidium* spp. and *Giardia* spp. The percentage of cases in which at least one pathogen was detected was increased from 53% to 76% through the use of molecular detection methods. The true burden of disease associated with rotavirus and norovirus was established and shown to be significantly higher than that caused by bacteria and parasites. In addition, a high proportion of enteric viruses, bacteria and parasites were detected in the cases as well as the asymptomatic controls. Norovirus and rotavirus were more commonly found in children while *Campylobacter* and *Salmonella* were detected in symptomatic teenagers and young adults, Enteroaggregative *E. coli* was found in all ages, and parasites in children (*Cryptosporidium* and *Giardia*) and young

adults (*Giardia*). Further the seasonality of infection with norovirus was autumn/winter, rotavirus was winter/spring, *Salmonella* and Enteroaggregative *E. coli* summer, *Cryptosporidium* was spring and autumn and *Giardia* was spring and summer.

The findings of project B14005 suggests that the burden of disease associated with viral infections was high and, although predominant in children, was found in all age groups. However, asymptomatic infection was not uncommon and may provide a substantial reservoir for onward transmission.

Main discussion points

Christine Dodd noted that the asymptomatic controls were demonstrating a high level of carriage and asked whether low levels of infection not presenting as disease was being observed or whether organisms were transient passive flora that was simply passing through the host not interacting with it. Jim Gray confirmed that it represented asymptomatic infections which are widespread throughout the population, particularly with viruses. In the first four years of life every child is probably infected with 2 or 3 rotavirus strains and several different norovirus genotypes and represents the reservoir within the population. These viruses were likely to have been passed onto parents and carers. If adults were not infected with any of these viruses in the recent past, then infection is likely to be symptomatic but if the virus has been seen in the recent past then an asymptomatic infection is likely. Dissecting this further, people who are having their first child are likely to have symptomatic infection whereas individuals with a 2nd or 3rd child may have an asymptomatic infection as a result of immunological protection from previous recent exposure.

Charles Penn mentioned that in doing a real time PCR assay looking at gene expression, if anything much above 35 cycles is used, false positive signals may frequently be detected. Charles asked what controls were being used and what the DNA concentration was in samples being tested. Jim Gray mentioned that positive controls, extraction controls and negative controls were used for each assay. It has to be taken into account that as many as 10^{12} particles per gram of faeces were going into the assay. High concentrations of these viruses are found in samples collected from symptomatic individuals with acute infection. Jim also added that DNA concentration was not measured during this study. Charles enquired whether using 40 CT cycles was indicative of something equivalent to a single virus. Jim was not sure if it is equivalent to a single virus but mentioned that data analysis is currently being carried out. Anything above cycle 30 is likely to be associated with no symptoms whereas amplification at a cycle below 30 would be found in a sample from a patient with symptoms. Jim McLauchlin (Health Protection Agency) also added that very high correlation was seen between the samples from which the *Salmonella* was only detected in enrichment and high CT values in the PCR whereas much lower CT values were observed for those cases detected directly by culture. The PCR test correlated with detecting very low target pathogen numbers.

Mike Carter asked whether the majority of the new norovirus infections being detected were genogroup 1. Jim Gray informed Mike that the HPA are currently working on the norovirus genotypes. The HPA are carrying out genotyping of the strains to determine their genotypes and whether that plays a significant role in viral load or whether we can take a 'cut-off', representing a symptomatic infection to be at cycle 30 - anything below

30 would be significant and anything above would not. Mike also asked for clarification on the age distribution of the asymptomatic cases. Jim mentioned that the age distribution of the asymptomatic controls was different depending on the virus. With norovirus, symptomatic and asymptomatic infection was observed in the whole population, whereas with sapovirus, asymptomatic infection was seen, mainly in young adults presumably caring for young children. So there is clearly a difference in biology between the 2 viruses.

Andrew Fox (Health Protection Agency) asked the speaker to comment on what appears to be target specific ability with respect to *Giardia*. Jim Gray suggested that this may be a concentration issue as little DNA is present in the sample resulting in a lack of buffering. Jim thought that either the DNA was being damaged when cell bursting was attempted to release the parasite nucleic acid or that the process was not efficient enough to release the entire DNA. Very low concentrations of DNA or cDNA will tend to dissipate very quickly, whereas, higher concentrations seem to have a carrier effect. It was not determined during the study whether this was the case.

Andrew Fox asked whether there was any corroboration of asymptomatic infection in the control population in terms of sero-epidemiological research. Jim Gray confirmed there was a link. For example, it is rarely found that genogroup 1 norovirus is associated with outbreaks in the population or even sporadic cases but 95% of the population have antibodies to genogroup 1 at a very early age (at around 10 years old). This is the reason why the control group was so valuable.

Will Waites enquired if bacterial spores were being detected. Jim McLauchlin clarified that none of the targets work for bacterial spores. *Clostridium perfringens* was looked for in the pilot study but was not taken forward into the main study. DNA is present on the outside of spores and it is difficult to determine whether the DNA being detected is from the inside or outside of the spores. Extracting DNA from the outside of the spore is much easier and this is an issue that was not fully resolved.

David Tompkins (Health Protection Agency) commented that the presentation and subsequent discussion suggest that norovirus were all sporadic cases in the community and that type 1 cases are very rare. Were the norovirus sent to the HPA reference laboratory from sporadic cases as well as outbreaks? Jim Gray mentioned that the HPA have conducted studies in the community for norovirus in the past; for example the HPA has recently completed a large nappy study looking at norovirus. The study showed that the most predominant strain in the community was GII-3, even though GII-4 is the most predominant strain in hospitals. So noroviruses are definitely present in the community but this does not mean that it has to be genogroup 1. Genogroup 1 noroviruses are found in samples collected from asymptomatic individuals in the community but not to the extent that the sero-epidemiology reflects. It is similar to rotavirus group C, where 30% of the population are carriers but symptomatic infection associated with Group C rotaviruses is rare.

Diane Newell asked what difference the findings of this project will make to our understanding of all the causes of community cases. In other words how much further we are in getting towards 100% identified agents from the 50-60% detectable in the first IID study by culture? Jim Gray stated that it is possible to identify an aetiological agent in up to 70% of the population. The data tell us that ways of measuring whether the organism is actually causing disease or an asymptomatic infection needs to be found.

Mike Gasson asked to what extent the provenance of infection for a particular sample was determined i.e. was it known if these were foodborne infections as distinct to other gastrointestinal tract conditions. This is relevant as the symptoms being presented could be caused by some other infectious agent or by a non-infectious disease state (e.g. inflammatory disease associated with immune systems dysfunction). Jim Gray responded that this is dependent on the case definition which was set up at the beginning of the IID study. The case definition was defined as multiple episodes of diarrhoea and vomiting within 24 hours of which there many. Mike commented that you would not expect to find pathogens in all cases. Jim agreed and noted that a limited number of pathogens and viruses were included in this study compared to those that cause IID.

Project B18021: The second study of infectious disease (IID) in the community - determining disease burden and calibrating national surveillance data in the United Kingdom

Professor Sarah O'Brien, University of Manchester

The contractor's B18021 project summary is included in Appendix 22.

The aims and objectives of the IID2 study are to: estimate prospectively the number and aetiology of cases of IID in the population contacting NHSD/NHS24, presenting to GPs and having stool specimens sent routinely for laboratory examination in the UK and to compare these numbers with those captured by the UK laboratory reporting surveillance systems; to determine the proportion of cases of IID likely to have been acquired abroad; to compare the surveillance pyramids from the first and second studies of IID for England; to estimate the number of cases of IID in the population of each UK nation, based on recall, via a national telephone survey of self-reported diarrhoea, which will be conducted over two time periods (seven days and four weeks); to compare the burden of self-reported illness captured through the national telephone survey with the burden of self-reported illness captured through NHSD in England and NHS24 in Scotland; to compare the prospective and self-reporting methods for estimating IID incidence in the UK, over two time periods: seven days and four weeks..

The IID2 study is ongoing but the implications of the pilot study are that the research methodology works, although there is lower than expected participation in the prospective cohort study and invitations from GPs for patients to take part in the presentation study. Some other minor issues became apparent during the pilot study and protocol changes have been made and implemented for the main study.

Main discussion points

Tom Humphrey asked whether there were any differences between the individuals who volunteered to take part in IID2 and those who didn't. Sarah agreed that people who take part in research tend to be slightly different to the rest of the population. One of the things this study should be able to do is to look very carefully at the characteristics of the population who take part in IID2 so that the characteristics of the cohort in this study can be compared to the general population. There are obvious differences in people's motivations and this was probably true for the IID1 study as well.

Mike Carter mentioned that a decrease in foodborne disease was cited as an anticipated outcome which prejudices the findings of the survey (which in fact is simply looking for a change) i.e. that there might be an increase. Sarah clarified that she was looking for a change but the Food Standards Agency would prefer to see a 20% reduction. The sample size is sufficient to allow detection of a 20% change (decrease or increase) in severe disease if this has occurred.

In light of the work in Denmark on sero-surveillance pyramids, Diane Newell wondered if Sarah had considered getting the serum from some of the cohort from the IID2 study. Sarah mentioned that in Denmark, studies have looked at serum for evidence of *Campylobacter* and *Salmonella* infection to try to get an idea of population exposure by looking at population infection as opposed to disease. Obtaining some of this data was considered but the funding received would not allow for this. There are also scientific considerations with this approach as it would involve obtaining blood samples which might affect participation rate. Furthermore there would be ethical considerations around obtaining blood samples from children.

Dominic Mellor asked if a third IID study is avoidable, given this is the second study and should hopefully show some sort of trend from first study. Sarah pointed out that there needs to be a minimum of three points on a trend line. Rather than repeat the whole study it might be possible to just repeat certain parts of the methodology and part of the aim of the IID2 study is to find out if this is possible.

Will Waites asked how sensitive the detection of toxin is as opposed to detection of DNA. Sarah asked her microbiological colleague, Henrik Chart, to respond. Henrik suggested that the sensitivity is dependent upon the toxin being detected. Verotoxin detection is extremely sensitive but the more dubious toxins such as peptide-toxin in *E. coli* is very difficult to detect.

Dominic Mellor highlighted that one of the main aspirations of the FSA in this area is to look at geographic differences. Assuming that this study involves conducting a UK wide study, how much are explicit spatial hypotheses incorporated in the sample size requirements? Sarah explained that they had looked very carefully at the geographical differences in the first IID study in England and essentially there was not a huge amount of variation across the country. To a large extent the geographical element of the IID2 study is governed by where enthusiastic general practices can be found. A large amount of information is gathered on the characteristics of these practices and each practice's population and compared with general practice as a whole in the UK. This will enable results to be adjusted during analysis. Currently, the cohort is slightly under-representing affluent people and urban areas but this is beginning to even out as more practices come on board.

Thomas Quigley (Safefood) asked if there was a food attribution part of the study and if it was based on the FSA 5 target microorganisms, or was there more work being done on the whole of food attribution. Sarah confirmed that the study is looking for the Agency's 5 target pathogens but also other viruses, bacteria, bacterial toxins, *Cyclospora*, *Cryptosporidium*, sapovirus, norovirus, etc. In terms of how this study may be of use in the future for food attribution, one of the things that is not covered by the IID2 study is food-related risk factors as this was unsuccessful in the first IID study. Reasons for this were firstly that a one-size-fits-all questionnaire was used in the IID study and for pathogens that have an incubation period of 30 minutes to 3 weeks

defining the appropriate exposure window was very difficult. Secondly the IID study data on individual pathogens were sparse because the numbers detected were small - there were only two pathogen-specific case-control studies, on *Campylobacter* and rotavirus. So looking at individual pathogens for food specific risks is very difficult even in a study of this size. However, IID2 study data combined with outbreak data might be used to study food attribution.

Thomas Quigley mentioned that Safefood has also used telephone randomised digital dialling but this was of limited success. Thomas asked how Sarah intended to target the younger generation who use mobile phones and is a section of the population that will be underrepresented. Sarah explained that they did give careful consideration to using mobile telephone numbers in the random telephone digital dialling strategy but could not due to ethical concerns about making unsolicited phone calls to children without their parents' consent. It is possible that the young males (15 to 25 year olds) in the cohort will be underrepresented in the study, but this is common in epidemiological studies.

OPEN DISCUSSION SESSION INCLUDING FUTURE PRIORITIES IN THE AREA OF EPIDEMIOLOGY OF FOODBORNE DISEASES

Chairman: Professor Bill Reilly

Bill Reilly introduced this session and informed the audience that the purpose of this session was two fold. Firstly, to determine how well the research presented on day 1 met the original research requirement (provide in the B14 foodborne diseases research review delegate packs) and secondly, to identify what the research gaps are and what the future priorities should be.

Did the research address the research requirements?

Bill Reilly invited the audience to comment on whether they thought that the epidemiological research presented on day 1 addressed the research requirements.

Peter McClure (Unilever) did not believe that antimicrobial resistance was adequately addressed in project B14001 as there was a lack of information on antimicrobial resistance other than whether we got a *Campylobacter* resistant or sensitive strain. Eric Bolton (Health Protection Agency) emphasised that a lot of work is being funded on antimicrobial resistant *Campylobacter* throughout the food chain and to comment on the results of a single study (B14001) without the rest of the body of work being incorporated risked taking this out of context. Project B14001 was investigating the incidence or prevalence of resistance in human disease particularly in travellers.

Bill asked the delegates if the impact of foreign travel and its effects on antimicrobial resistance should remain a priority area for the FSA. Tom Humphrey was unsure what could be done with the data if a study was funded. Tom accepted that you can calculate the burden of foreign travel but was undecided on what the FSA would do once this information is available.

Celia Caulcott (Cooper and Caulcott Ltd) emphasised that there has been a dramatic change since the first foreign travel project was established. Foreign travel in the UK has radically changed and increased so the question is really on antimicrobial

resistance and whether we need to know the impact of foreign travel on infectious disease.

Thomas Quigley mentioned that from the perspective of Safefood it provides you with the evidence base to develop policy and communications programmes for information for travellers and for other Government departments. Bill agreed but reminded delegates that this is not the role of the FSA but other Government Departments.

Bill asked the delegates to comment on whether the AEGISS project has taken us forward.

Charles Penn suggested that the AEGISS project highlighted the difficulty in obtaining good data from primary care and if the intention was to persist with this approach careful deliberation is needed on how to make it work.

Sarah O'Brien suggested that one of the most useful things about AEGISS was the incorporation of new data streams that had not previously been examined in terms of trying to give an assessment of both burden of IID but also to detect outbreaks earlier. Sarah added that she would be interested to know what the HPA decided to do when the AEGISS project came to an end. It begs the question of how new or potentially new surveillance systems are assessed and introduced and whether there is a strategic process for adoption of new schemes once research funding ends.

Peter Hawtin explained that current data protection laws are fraught with problems. The project was carried out during the period when the PHLS was decommissioned and the HPA formed. The project did find favour with the emergency planners with the threat of bioterrorism and the increase of detecting respiratory agents more rapidly. The technology is catching up with the approach and the foundations are being laid for better quality, more consistent data streams, which can be used to detect emerging problems more rapidly.

Charles Penn enquired whether there was the potential to improve the mathematical side of the AEGISS project as there seems to be fundamental work that needs to be completed to get the spatial modelling better established and this is probably an aspect of progress that can be built upon.

David Tompkins stated that the problems that AEGISS and other surveillance reporting systems in England have is that they are based on voluntary reporting from clinical systems rather than having specifically set up surveillance systems. Meirion Evans said that there are sentinel spotter practices in Wales but also bigger schemes elsewhere in the UK for example the Royal College of General Practitioners Scheme and Q Research, which is a large multiregional scheme based in Nottingham. There is also viral influenza surveillance in winter based on spotter practice schemes. Meirion also added that plugging into the NHS Direct had enormous potential and if it was taken further would be automated.

Peter Hawtin also mentioned that he was unable to cover the power of Gastrointestinal Infection Surveillance (GIS) in his presentation and show that it was possible to the spatial statistics with microbiology and with geographical features such as with reservoir layout and distribution zones. The contribution added to the framework of spatial statistics is largely unlimited using GIS. All the data gathering in the project was

automated. The data would come in, be cleaned, de-duplicated and transferred to Lancaster University, where the model was run over night. So the speed of the turn around time was not human dependent.

Meirion Evans made a comment on NHS Direct. There is an automated system where the data from NHS Direct is used to track gastro-intestinal symptoms but the usefulness will depend on the objective of the surveillance. If you want surveillance to monitor trends of infection for example against an FSA target then you may come to a different type of system to one which is intended primarily to identify clusters. So you need to be clear what the objectives are first and tailor the surveillance scheme to meet your objective.

Andrew Fox explained that the presentations provided today demonstrate the power of using molecular detection methods because there is always a syndromic diagnostic approach. Syndromic surveillance can also be carried out along with pathogen screening which increases sensitivity. This adds another dimension in terms of investigating IID.

Jim Gray made the point that the HPA have worked with NHS Direct on norovirus, so when the number of cases in the community in young children start to rise, the start of outbreaks can be predicted the location of these outbreaks cannot be identified.

Bill Reilly asked the audience to provide their thoughts on the two nucleic acid projects presented in the afternoon and how far they went to meeting the requirements. Bill felt that creating a pool of material is common sense but there are questions on ethics and the difficulty in archiving faecal samples.

Eric Bolton agreed that there will definitely be a problem with archiving human faecal samples in the future as the human tissue act embraces faecal material as part of it. In order to store and share this material we need to make sure the right ethical approval and consent is obtained for future research. This needs to be factored into research requirement bids the FSA puts out using faecal material.

Will Sopwith raised a word of caution in that the longer the period of time faecal material is archived the greater the disconnection with the epidemiological context in which the sample was collected and this may lead to all sorts of assumptions and/or misleading conclusions from later analysis. It is important to collect a really good epidemiological dataset along with the faecal sample collections but it is unclear whether this was addressed in the original IID study.

Sarah O'Brien confirmed that the archive generated from the IID study includes the epidemiological data collected during the study. This is archived at the University of Essex and is freely available on the University of Essex website. The IID2 study team have explicitly asked for consent to store stool samples for future research and the epidemiological information generated will also be archived.

Bill Reilly stated that it was difficult to judge the IID2 study against its objectives as the project is ongoing, but the presentation indicates that there are several studies in the project and it would appear to be more successful than the original IID study. Overall there seems to be a general consensus that the projects went well but for each one there are areas which could be addressed further by the Agency.

Thomas Quigley brought to the attention of the audience that an IID2 study is being carried out in Ireland which includes both Northern Ireland and the Republic of Ireland. SafeFood were frequently asked by the researchers about the devotion of large sums of money to the IID2 study and the lack of information about attribution. The IID2 study is a major study which covers a lot of public health information that is not specifically food related and it appears that there is the devotion of large sums of the FSA research money to the project that could be joint-funded by some other funders. Could the FSA comment? Tom Humphrey also added that it would be interesting to know how much of the Agency budget is divided between research and surveillance.

Gael O'Neill suggested that 51% of the Agency's research budget is spent on food safety issues but was unable to provide further details on the split according to research or surveillance at this time. Further information should be available on the Agency's website.

Sarah O'Brien clarified that the total cost of the IID2 study is £5.5 million of which £4.1 million of the research cost is covered by the FSA, with another £1.4 million provided by the Department of Health in terms of NHS service support costs. So there is an element of budget sharing with this project.

In response to Thomas Quigley question, Paul Cook said that in the first IID study there was an attempt to identify food sources for some infections but that this was only partly successful as, in some cases, the numbers were small. Using the IID2 study directly to derive food attribution would be very difficult (there is no control arm to the study). However, the study will provide information to inform the development of more robust attribution studies and the Agency sees this very much as underpinning its long term monitoring of trends in foodborne disease. The Agency regards the IID2 study as important but recognises that not all IID is foodborne. It should be mentioned that the study does receive service support costs from the Department of Health. Ultimately, it is important that the study is taking place and it will take us forward in understanding not only IID but hopefully in subsequent work, food attribution as well.

Thomas Quigley commented that between 20-30% of IID is thought to be due to food and water sources but the FSA is paying 80% of the budget for the IID2 study.

In defence of the FSA, Celia Caulcott pointed out that there is a whole series of resources (like the IID study) which are being funded by different parts of Government and research councils in the UK. Any one body may well fund something of value to many government departments and other bodies, and equally benefit from the work supported by other departments. The FSA are contributing to a very valuable set of data which will be used by many such as the BBSRC, Defra and DH for similar activities.

Meirion Evans argued that you could divide the costs not only on the percentage attributed to food but also according to the seriousness or number of fatalities which would probably swing the balance towards foodborne disease.

Andrew Fox thought that the IID2 study will create a valuable resource because the study will produce a lot of information on source attribution. The study will incorporate information generated from other studies that the FSA are funding, particularly in terms

of strain typing and applying the technology that will be available and the material from the IID2 study will contribute greatly to source attribution.

Gaps in research and future priorities for the FSA in the field of epidemiology

The delegates were asked by Bill Reilly to concentrate on the gaps in research and to identify the future priorities the Agency should be considering in the field of epidemiology.

Tom Humphrey stated that we know that people travel abroad, become ill and return to the UK. However huge amounts of food are imported into the UK which has the potential to cause food poisoning, such as Spanish eggs. Tom would endorse further investigations of food attribution in UK produced foods against imported foods. Such a study would give a split of imported foods causing disease and indigenous food causing disease.

Dominic Mellor pointed out that there seemed to be a big gap by not incorporating some parallel food surveillance for the same pathogens at the same time as the IID2 study is gathering data on human illness. Dominic thought that if the study was going to run over a period of time and there are various cohorts of the study moving along, it seemed to be a missed opportunity not to be looking for what is in the food at the same time, even if the two cannot be directly linked – it would provide information on what the population is going to be exposed to through food. Jim Gray added to Dominic's comment that the surveillance needs to be syndromic.

Jim Gray continued by suggesting that the Agency needs to be looking at viruses as well as bacteria because there is a tendency to ignore viruses. When viral foodborne outbreaks are investigated, there is a tendency to find multiple viruses associated with those outbreaks and these are a good indicator of faecal or sewage contamination.

Meirion Evans pointed out that *Campylobacter* remains the key issue as it is the biggest contributor to foodborne disease but only 50% of those infections can be attributed to poultry while the source of the remaining 50% is unknown. There is still a big gap in terms of foodborne sources for *Campylobacter* infection. There is a continued need to look at the epidemiology of *Campylobacter* in terms of aetiology of infection and linkages to food surveys or trace back to farms.

Dominic Mellor suggested that an exciting part of this research is the case definition issue. Dominic agreed that there has not been enough work investigating viruses and there is also a lack of information on co-infection.

Andrew Fox commented that there is a huge amount of information that has been gathered, the majority of which has been funded by the FSA and Defra. Some of the issues around source attribution, importance of *Campylobacter* in poultry and particular strain types is available in these studies but they need to be utilised. Diane Newell supported Andrew's comments but added that this should not be limited just to the UK as there is considerable data available from across Europe that many UK institutes have been involved in generating but this data is not being utilised appropriately. Diane also added that original IID study generated an impetus for foodborne disease research worldwide but the data from this study look a long time to publish and become publicly

available. She would, therefore, like to see the IID2 study data published and being feed into the B14 research programme as soon as possible.

Jonathan Fletcher (University of Bradford) noted in the presentation on traveller's diarrhoea that the strain association with food is not really a UK problem and the FSA can not be responsible for what individuals choose to consume abroad. However, these travellers do import bacteria and viruses into the UK. Therefore it would be interesting to learn how easily these strains, particularly the antimicrobial resistant strains, establish themselves within the UK pool of potentially pathogenic isolates. If these strains are not established, what is the potential for antibiotic transfer through horizontal gene transfer?

Thomas Quigley suggested that in the future the FSA could be strategic in terms of evaluating surveillance methodologies and assessing what are the best methods to get information more rapidly; whether it is food attribution information or human surveillance issues. Factors such as the best, cheapest and most effective would be useful for both food and human surveillance.

Tom Humphrey mentioned that there has been a recent upsurge of listeriosis in the over 60's. There is good information on the quality of the food that leaves factories but there is a lack of information on consumer practices in the home. A recent North West study on stored cold cooked meats illustrated that current food surveillance for *Listeria* may not be sensitive and may miss low levels of contamination. It is still unclear what the level of consumers' knowledge on food safety practices is and what they do with their fridges. The Agency needs to target advice to the appropriate groups based on what is occurring in their homes. Gael O'Neill responded that this is an area of research that the Agency is addressing through its B20 domestic sector hygiene research programme.

Charles Penn commented that knowledge on microbial genomes is very rapidly increasing and that there will be new molecular markers and new insights into microbial diversity. This might help, for example, with the question regarding *Campylobacter* speciation that arose in discussions earlier. Charles was concerned that the cost and difficulty in collecting these archives of material is huge and that the amount of DNA obtained from these archived samples is crucial if their value is to be maximal. It would be good to think that it would be possible to re-visit the archive in future years to look for new pathogens that are not recognised at the moment but this is dependent on whether there are sufficient amounts of DNA. These types of technical questions could be important in the future.

Mike Gasson suggested that the Agency could address the gap between pathogen detection and disease symptoms. This important study is relevant to the food chain in terms of food producing animals, relevant in humans in the IID study and has broader potential relevance to gastrointestinal tract health in general. This is probably not the responsibility of the FSA but is an interesting side question to do with the totality of gastrointestinal tract health status and susceptibility to disease, which brings in total microflora. For example it would be interesting to carry out global microflora analyses on some of these samples.

Sarah O'Brien supported Mike's comments on the interaction between normal gut flora and pathogens but added that samples are only being collected in sick people in the

IID2 study. It would be preferable to gather samples from these people before, during and after the episode of food poisoning.

Diane Newell suggested that human host susceptibility has not been effectively covered in the area of intestinal infectious disease epidemiology, particularly in relation to campylobacteriosis. It would therefore be very valuable to investigate the effect of human protective immunity and its effect on the source attribution, disease trends and risk assessment of IID. This area is now generating considerable interest, especially in Europe, and should be an area the FSA should investigate in the future.

Eric Bolton claimed that there is a tendency to concentrate on the acute phase of the diarrhoeal disease as part of the disease burden and suggested there is a lack of focus on the sequelae of these infections and the burden that brings to the NHS and individuals. Meirion Evans indicated that some aspects of sequelae were investigated in B14001 but this remains a huge gap in this area.

Sarah O'Brien commented that the World Health Organisation have put together an international group to work out the global burden of foodborne disease and are taking sequelae into account. They are also looking at disease syndromes outside the gastrointestinal tract such as *Listeria* meningitis and *Salmonella* meningitis as well as diarrhoeal diseases.

Bill Reilly added that some published evidence suggests that toxoplasmosis is a very significant cause of illness in society and should therefore be included to plug a potential gap in research. Will Waites also added that new and emerging pathogens including *Listeria monocytogenes* should be included as a priority.

Prioritisation of the research area identified

The future research priorities identified during day 1 open discussion session were:

- Imported against UK produced foods
- Enhanced food surveillance during the IID2 study
- Looking at syndromic work on viruses and bacteria
- How antibiotic resistant strains establish in the UK
- Further investigations on the epidemiology of *Campylobacter*
- Revisit case study definitions as subset of other work
- Better mining of existing data bearing in mind data that is still current
- Putting the IID2 study data back into the system as quickly as possible
- Evaluating surveillance systems
- Addressing gaps between pathogen detection and disease outcome
- Host susceptibility including the role of immunity
- Sequelae
- Toxoplasmosis
- New and emerging pathogens

Bill Reilly asked the delegates to try to identify 3 or 4 areas from the list above that the Agency should be addressing in the future.

Sarah O'Brien gave her support for a risk based cost effectiveness study which takes into account chronic sequelae and acute infection. Eric Bolton agreed but suggested

that any work carried out on sequelae should be joint funded by the FSA and the Department of Health as a major part of the study would be clinical.

Tom Humphrey offered his support to host susceptibility because we have an aging population that is becoming more vulnerable and foodborne disease rates are increasing in this group. There is a need to know why certain individuals are more susceptible to disease compared to others. Diane Newell agreed that host susceptibility should be a top priority for the Agency. At the moment all risk assessment models assume that every host is equally susceptible to foodborne disease which is clearly not the case. Further work in this field will not only improve our understanding but also improve our risk assessment models.

SESSION III: REVIEW OF THE FSA'S B14 *CAMPYLOBACTER* AND *SALMONELLA* RESEARCH

Project B14011: Case-control study of risk factors for *Campylobacter jejuni* infectious intestinal disease (IID) in England and Wales

Professor Sarah O'Brien, University of Manchester

The contractor's B14011 project summary is included in Appendix 20.

Project B14011 aims were to: identify risk factors for human indigenous sporadic *C. jejuni* IID and to determine the proportion of cases of *C. jejuni* IID attributable to food and other exposures through the calculation of exposure specific population attributable factors; to investigate whether different risk factors are involved in the annual rise in incidence during late spring and whether the distribution of important risk factors varied by geographical region; to explore potential association between *C. jejuni* subtypes and routes of transmission, seasonality and geography and to evaluate genotyping as a molecular epidemiological tool for the differentiation of *C. jejuni* strains using a subset of human isolates of known clinical, epidemiological and phenotypic characteristics and finally to identify genetic determinants of *C. jejuni* that may be associated with different routes of transmission and severity of disease.

The results of all the component studies pointed to chicken-related exposures as a risk factor for a substantial part of human illness. Consequently reducing *Campylobacter* contaminated chicken entering the food chain should have a major impact on lowering disease burden. In addition, since eating chicken outside the home was strongly associated with the risk of illness, advice to caterers needs to be enforced. Analyses using MLST and comparative phylogenomics approaches can potentially help to refine case definitions by identifying subgroups of *Campylobacter* that are more epidemiologically relevant. Antacid use accounted for about 10% of all cases and advice to regular consumers of antacids is needed to reduce the risk of acquiring not only campylobacteriosis but other causes of gastroenteritis.

The analyses using MLST and comparative phylogenomics data demonstrate the potential for the combined use of genome-based typing methods and detailed epidemiological information for the study of *Campylobacter* and other pathogens.

Main discussion points

Mike Gasson asked Sarah O'Brien to explain why genotyping and MLST were used and the advantages of using these methods. Sarah explained that with *Campylobacter* typing in general it seems possible to achieve a great deal of discrimination no matter what method is used. As an epidemiologist Sarah was looking for a scheme that "lumped" things together in an epidemiologically sensible way and this study has shown that this is achievable. These methods were chosen partly because it was an ACMSF recommendation that MLST should be exploited as a laboratory tool and this was an opportunity to test out this recommendation. Secondly, comparative phylogenomics looked like it had the potential to offer this clustering as opposed to splitting, which seems to be characteristic of *Campylobacter* typing. The results were consistent with each other and probably useful but Sarah would not suggest that this is necessarily done again.

Charles Penn was intrigued by the focus on chickens, the thinking about consumers' dietary habits and the correlation between high levels of chicken consumption and high levels of meat generally. Charles asked for clarification on how many participants were vegetarians and whether there was a link between those eating pulses and being vegetarian or seldom eating meat of any kind. Sarah commented that although the reporting of people's diets was consistent, there were a lot of inconsistencies in the way people had reported vegetarianism. For example there were participants recorded as being a vegetarian but who had eaten chicken in the past 5 days. So the analyses were conducted including and excluding these people to look at the effect on risk. Other meats were also looked at but while some of those appeared as risk factors in a single risk variable analysis they dropped out of the multivariable models. Charles added that it is probable that the study picked up people who do not consume red meat but eat chicken and consider that to be vegetarian. Sarah explained that the study has demonstrated how difficult it is to power a study adequately when looking at very common exposures as well as very rare ones. Both sparkling and still bottled water were also investigated but no evidence was found in this study that bottled water posed a risk. Environmental exposures were also considered but they also dropped out.

Tom Humphrey asked Sarah if she could provide some information on the type of chicken people are consuming outside of the home. Sarah responded that most of the chicken being consumed from outside the home was from restaurants or takeaways but detailed description of the type of cooked chicken being consumed was not collated.

Christine Dodd was interested in the fact that both those who drank unpasteurised milk regularly and those that ate chicken more frequently had reduced risk in comparison to those who do not eat chicken frequently. A possible explanation could be some sort of immune response. Christine asked Sarah if she thought people should be eating less hygienically and exposing themselves to chicken. Sarah thought that this demonstrates that case-control studies are not necessarily the best method to investigate the risks for *Campylobacter* infection because there is generally not as much care taken in defining controls as there is in defining cases. A lot of immune and semi-immune controls tend to be included in case-control studies that do not have a chance in developing the disease, so whilst this evidence might be used in the argument to support the hygiene hypothesis, if there were genuine controls in the case-control study, the risk might have looked very different.

Dominic Mellor commented that half of the cases were attributed to chicken and then asked Sarah what she thought should be targeted next and whether she would look 'somewhere' else. Sarah thought that the first step would be to step back from population attributable fractions and consider whether it would be reasonable to think that 100% of cases in a case-control study could ever be explained using the current methodology. The answer would be 'No' because there might be other factors that had not been accounted for or asked about, that are potential risks for *Campylobacter* infection. So it would not be reasonable to assume that in a case-control study it is possible to explain 100% of cases. At the start of this study, the leading hypothesis was not related to poultry consumption but related to environmental exposures. Part of the aim of the study was to investigate the proportion of *Campylobacter* that could be attributed to food and the proportion of *Campylobacter* explained by exposure to the environment. This further illustrates the difficulties of designing a study with sufficient power to be sensitive enough to pick out rare exposures as significant.

Will Sopwith asked whether the clusters were identical in terms of genomic and MLST type and also what typing was used to identify them. Sarah O'Brien clarified that the clusters were identical MLST sequence types.

Ian Toth (Scottish Crop Research Institute) mentioned that the 2 different clusters (livestock and non livestock) were assessed using phylogenomics and asked for further information on the non livestock cluster. Sarah explained that for the epidemiological data collected on individuals, they tried to compare the exposure data from people with isolates in clade A with those in clade B using case-case analysis. However since the subset was so small there was insufficient power to detect statistically significant differences in risk. Also the datasets used for comparison with source isolates in Oxford and London School of Hygiene and Tropical Medicine are skewed towards poultry isolates as that is where the majority of the work has been done. So it is difficult to make any firm statement until the isolates in the datasets are from a more representative distribution of sources.

Ian Toth explained that his area of interest is with plants but it is difficult to find any studies investigating *Campylobacter* in plants. Did any aspects of project B14011 look specifically at salad and vegetables? Sarah confirmed that the study did not look at plants.

In response to a question from Andrew Fox, Sarah clarified that no separate analyses have been carried out on *C. coli*.

Eric Bolton commented that a lot of information was presented on poultry and hygiene in the kitchen and asked whether there was any association with increased risk with red meats and red meat products. Sarah confirmed that there was no increased risk from red meats.

Will Waites asked if chopping boards (wooden versus plastic) were investigated under this study. Sarah suggested that she would have to recheck the questionnaire but thought that chopping boards were not covered specifically under this project.

Celia Caulcott asked Sarah what approach she would now adopt if the study was carried out again as she had indicated that the case-control study methodology was not appropriate. Sarah thought that a case-control approach would not be helpful when

trying to investigate environmental sources for *Campylobacter* infection. A preferable approach would be to use the type of spatial analysis that has been successfully used for VTEC. This would involve employing sophisticated spatial statistical techniques to analyse data and overlaying this with other risk information from water sources, water course, ruminant density, poultry farms, etc.

Meirion Evans made the comment that there had been some discussion on the difficulties of getting appropriate controls, representative cases and biased subsets. Meirion asked Sarah whether she thought that these can have different risk factor profiles to those we know about and may have more severe symptoms that people are likely to present to their GPs. Sarah agreed that this was possible but added that in original IID study a case-control study was performed on cases of *Campylobacter* infection who have not gone through the selection processes described and these findings were similar.

Project B14012: Environmental and waterborne sources of *Campylobacter* in the North West of England and their influence on seasonal human infection
Professor Eric Bolton, Health Protection Agency

The contractor's B14012 project summary is included in Appendix 21.

A high proportion of human infections with *Campylobacter* remain unexplained. Incidence in the UK shows a marked seasonality and this may be mediated by environmental factors. Project B14012 intended to examine clusters in the North West of England and assess the contribution that environmental exposures, including water-borne sources, make to the human disease.

The study confirmed the utility of MLST for strain characterisation of *C. jejuni* and *C. coli* and its value for population based investigations of isolates from human and environmental sources. The major route of *Campylobacter* infections is foodborne transmission but some infections may be associated with environmental exposure. Environmental *C. coli* isolates in this study were different from those causing human disease and therefore the implication is that *C. coli* infection is almost always via the foodborne route. Imported infections contribute significantly to the burden of disease and whilst there is already evidence that resistance to quinolones is associated with foreign travel the study found that it is also related to certain clonal complexes. Recognition of *Campylobacter* outbreaks and clusters of cases proved to be difficult but the use of MLST in conjunction with active human surveillance can help to inform the investigation of epidemiology of infection.

Main discussion points

Tom Humphrey asked if anything was known about the biology of the different clonal complexes or serotypes. Eric Bolton confirmed that not very much is known because that is one area that has not really been studied. There is nothing, for example, comparing ST45 with the ST21 and looking at the strains survival characteristics etc. Will Sopwith commented that at the 2007 CHRO meeting in Rotterdam, a poster was presented from the Oxford Group on their work on ST45. They had looked at the resistance of different MLST types to freezing and ST45 did show greater resilience to being frozen than other *C. jejuni* subtypes.

Charles Penn noted that there were one or two comments about climatic events, and asked for clarification of whether this was one of the areas they were hoping to investigate as, in fact, the study only showed a seasonal correlation. Eric confirmed this and explained this was due to the fact that cases could not be linked to waterborne infection. It was predicted that there would be some seasonal event that precipitated *Campylobacter* coming through the water distribution chain. The organism was not detected in the water distribution system and therefore that link could not be made.

John Threlfall (Health Protection Agency) asked about ciprofloxacin resistance and whether the mutations were different in different strains, or was there one mutation common to all? Eric pointed out that ciprofloxacin resistance was not covered under this study. Andrew Fox confirmed that the HPA have identified some sequence markers, and have a correlation between different serotypes and antibiotic resistance but are still analysing the data. Andrew also explained that there is work being done at Liverpool as part of the Bristol Genomic Research Institute (BGRI) programme doing some comparative genomics which is taking some of the clonal complex representative, also referred to as ancestral strains, of those sequence types and doing some comparative genomics.

John Threlfall mentioned a recent paper that suggested that at a genetic level *C. jejuni* and *C. coli* appear to be merging and asked if there was any significance in that? Eric noted that there is certainly an argument from Oxford that *C. coli* is a precursor for *C. jejuni* and there are strains of *C. jejuni* that contain a mix of alleles usually only seen in *C. coli*. Eric thought that this represented the normal genetic diversity which is expected in any population of organisms and could not subscribe to this theory at this point in time.

In terms of the environmental aspects, Will Waites asked if the investigation looked at food processing plants. Eric revealed that food plants were not included as part of the study and stated that there is no such thing as environmental *Campylobacter*. There is *Campylobacter* isolated from the environment and this is linked to issues raised before in terms of survival etc. It would have been useful to assess food contemporaneously but that was not part of the funding received.

Alan Godfree (United Utilities) made the observation that the finding of *Campylobacter* in river water may just mirror what is occurring in the human condition because rivers receive discharge from waste water treatment works, and so the organisms may end up in the environment. Alan also made a comment on the initial hypothesis, particularly in terms of a seasonal link between *Cryptosporidium* and *Campylobacter* infections. When cases are plotted and compared with seasons it is evident that a rise in these infections occurs every year at approximately the same time and there seemed to be a relationship. However, from the late 1990s cryptosporidiosis outbreaks were associated with animal reservoirs as all the strains were *C. parvum* and not *C. hominus*. This seems to indicate that completely different epidemiological set of circumstances between *Cryptosporidium* and *Campylobacter* were being observed. That seasonal factor is coincidental and cannot be linked and this study demonstrates this to a greater extent. Eric indicated that the hypothesis was formulated at a time, when this body of knowledge was not available.

Diane Newell made two comments. Firstly, the VLA have been doing quite a lot of work on looking at physiological properties of individual strains of *Campylobacter* particularly in terms of survival in the environment. These isolates are all MLST typed and we now have a wealth of knowledge of comparative physiology from a large number of organisms. Secondly, with regard to B14012 presentation in which Eric mentioned *C. coli* was the predominant species in water related fowl, Diane wanted to remind the group that it is also a dominant species in sheep, and asked how much of that rural environment in the study environment was sheep-related run off? Eric agreed and confirmed that the study area was rural farmland which is a mixture of cattle and dairy farming and sheep. *C. coli* are the predominant *Campylobacter* in pigs but as the *C. coli* isolates from pigs are totally different to those found in humans the question of whether these are non-pathogenic arises. *C. coli* are not seen to be a major source of infection in humans but are coming through other parts of food chain be it poultry or other aspects.

In response to suggestions that human cases of campylobacteriosis could be definitely attributed to certain veterinary sources on the basis of MLST type, Diane raised a point of caution as the strains typed to date and described in available databases tend to be skewed by non-structured sampling. Although the MLST route is sensible the MLST databases must be looked at for continuity compared with national strain collections. For example, Defra has large collections of *C. jejuni* and *C. coli* from structured national surveys in cattle, pigs and sheep that have never been typed. Defra is also currently carrying out a national baseline survey for *Campylobacter* in poultry.

Project B03013: Comparison of the incidence of *Campylobacter jejuni* and *Campylobacter coli* in North West of England and Northern Ireland

Professor John Threlfall, Health Protection Agency

The contractor's B03013 project summary is included in Appendix 8.

The main aims of B03013 were the harmonisation of methods for the subtyping of *C. jejuni* and *C. coli* in Northern Ireland and England in order to study differences in the epidemiology of the organism. In addition, pulsed field gel electrophoresis (PFGE) was evaluated as a shared resource.

The application of serotyping demonstrated that while a large number of serotypes were observed, the majority of isolates from cases of human infection in Northern Ireland, North West England and Lothian belonged to a limited number of different types. In addition, a similar phenomenon was observed for phage typing. However some significant differences in serotype and phage type distribution were found between isolates from the three different regions. Variations in the structure of the food chains in these regions might be an explanation. For example, food is produced and consumed locally in a relatively closed food chain in Northern Ireland whereas in North West England food may be more likely to be contaminated with a variety of *Campylobacter* types from imported foods. The size of the catchment populations sampled in each area varied and may have produced differences in the reported frequencies of serotypes and phage types in some areas. Findings from the PFGE harmonisation study suggested further standardisation of methodology is vital before the technique is sufficiently robust for inter-laboratory comparisons suitable for epidemiological investigations. However, it would seem that broad based inter-laboratory comparisons

using standardised molecular methodology for subtyping may prove useful when linked to detailed epidemiological studies.

Main discussion points

Tom Humphrey asked how many people worldwide are using MLST typing compared to PFGE. John Threlfall replied that PFGE is the method widely used internationally for subtyping *Campylobacter*. For example CDC uses PFGE for typing *Campylobacter* and *Salmonella*, etc if there is a possibility of an outbreak. MLST is useful for long term studies, but for outbreaks and shorter term studies PFGE is more generally used

Mike Gasson commented that there were a few examples where PFGE results from the 2 laboratories in the study differed and asked if the reasons for this were explored. John confirmed that the discrepancies were investigated and repeated. John thought that the discrepancies could have been caused by the Queen's University of Belfast group overloading the gels with too much DNA whereas the HPA Colindale achieved more discrete bands by using less DNA. The DNA extraction procedure should have been fully investigated at the time to precisely identify where the problem was. Mike also asked if all the gels used the same enzyme and if there was capacity to use different enzymes. John confirmed that all the gels were based on results with the same enzyme, but that different enzymes would have to be used if there was an outbreak investigation or for real-time typing.

Bill Reilly asked how widely PFGE is being used in UK for routine typing of *Campylobacter* isolates and if there are plans to bring *Campylobacter* into European PulseNet. John confirmed that short sequence typing of the short variable region (SVR) of the flagellin gene and PFGE is routinely used at the HPA Colindale, particularly for outbreaks. John also confirmed that to his knowledge there are plans to incorporate *Campylobacter* into PulseNet Europe as and when necessary.

In response to Bill and Tom's questions, Diane Newell commented that PFGE is widely used. Diane pointed out that a standardised procedure for typing *Campylobacter* using PFGE was established in 2000 within the EU-funded Campynet project which was started in 1997. This project also provided an international *Campylobacter* strain set for harmonisation and standardisation of molecular typing methods. This strain set has been used by CDC and PulseNet. MLST is an incredibly valuable technique for looking at the evolution of populations of *Campylobacter* but PFGE is preferred for routine epidemiological studies especially when connecting strains from one environment to another, such as poultry environments around a farm. PFGE seems to be more sensitive for tracking strains in smaller time frames than MLST. Diane thought that PFGE is the more routine technique used worldwide because MLST is too expensive and more difficult for many laboratories. The disadvantages of PFGE, as for other molecular typing techniques for *Campylobacter*, are that the genome of *Campylobacter* is unstable. Under certain conditions, for example environmental stresses, the PFGE profiles can change because *Campylobacter* can shift its genome around. The organism is also naturally transformable and can take up foreign DNA. Also a proportion of strains are *Sma1* resistant so *Kpm1* is used as the second enzyme, but *Sma1* strains are clonal so they are quite interesting. All other molecular typing methods for campylobacters are also susceptible to the genetic instability of the *Campylobacter* genome through a variety of mechanisms.

Eric Bolton asked John for his thoughts as to why PFGE has not been adopted as 'THE' typing technique for resolving most of the issues with foodborne organisms and disease in the UK. PulseNet works in the USA but why has it not been taken forward in the UK for a whole raft organisms? John stated that PFGE has been adopted globally and has been used to identify outbreaks internationally. For example, there are international databases for *Salmonella* and profiles can be exchanged electronically within seconds. As a hierarchical scheme for *Salmonella* subtyping, there is serotyping, phage typing, resistance screening, plasmid profile typing and PFGE. Eric disagreed with John and suggested that PFGE has not been rolled out in the UK and there is currently no common platform for investigating all outbreaks across the UK.

Project B03014: Whole genome analysis fluorescent AFLP - identification of post speciality genes of *Campylobacter* and their value as epidemiological markers

Health Protection Agency

Professor Bill Reilly informed the delegates that the Health Protection Agency was unable to send a representative to present the findings of Project B03014 or provide a project summary. However the project was assessed by the B14 Expert Panel.

Further information on Project B03014 is available on the FSA's website at: <http://www.food.gov.uk/science/research/researchinfo/foodborneillness/foodbornediseasesresearch/b14programme/b14projlist/b03014proj/>

Project B01013: Genotypic subtyping of multiresistant *Salmonella* Typhimurium DT 104 from food, animals and humans

Professor John Threlfall, Health Protection Agency

The contractor's B01013 (FS3114) project summary is included in Appendix 7.

The main aims of the study were to standardise and apply recently developed molecular methods to sub-divide multi-drug resistant (MR) strains of *Salmonella enterica* serotype Typhimurium definitive phage type (DT) 104 (= MR DT104) and related phage types for analysis by pulsed field gel electrophoresis (PFGE).

Analysis by PFGE of 853 isolates of MR DT104 resulted in the identification of 41 distinct pulsed field profiles within the phage type, with 89% of the isolates being profile type Xtm 1. Eighteen of the remaining profiles were represented by a single isolate, the second most common profile accounted for only 2% of the total.

Overall the project demonstrated the applicability of molecular subtyping to outbreak investigations MR *S. Typhimurium* DT104 and emphasised the importance of a multi-method approach. The study also demonstrated the need for and use of standardised methodologies between the HPA and the VLA for outbreak investigations of *Salmonella*, as well as the importance of rapid and accurate transmission of electronic data between the two organisations. In addition, the study emphasised the need to continually assess new methodologies and their applicability for outbreak investigations and that a holistic 'farm to fork' approach to molecular epidemiology is very important for the investigation of outbreaks of bacterial foodborne zoonoses.

Main discussion points

Charles Penn asked John for his views on next generation sequencing and costs involved. John Threlfall confirmed the HPA are exploiting Variable Number of Tandem Repeats (VNTR) fingerprinting for *Salmonella* Typhimurium and several other *Salmonella* serovars. VNTR typing is cheap, rapid and efficient but the method requires international standardisation.

Yvonne Boyd (Department for Environment Food and Rural Affairs) asked whether any transposon like features were identified around Salmonella Genomic island -1 (SGI-1) that could account for it spreading. John revealed that SGI-1 has two integron sites and can excise and integrate into another strain. This has resulted in the island becoming transferred horizontally to several other serovars. Sequencing of the resistance genes within SGI-1 has revealed that some genes may have originated in bacteria other than *Salmonella*. For example resistance to tetracyclines was mediated by *tetG*, previously common in *Vibrio cholerae*. Similarly the *bla_{carb2}* ampicillin resistance gene had been identified in *Pseudomonas aeruginosa* from the Far East and did not develop in the UK. These results suggested that the resistance genes within SGI-1 were acquired by the organism before it became common in the UK. A possible origin for these genes was an aquatic environment and multiresistant DT104 may have arisen as a result of gene transfer in aquaculture in the Far East, resulting in a reservoir of MR DT104 in exotic or ornamental fish. Evidence suggests that the organism may then have been brought into the UK via exotic birds, spread to the wild bird population and then started infecting bovine animals. The use of antibiotics ensured that the strain became established in the cattle population and it subsequently spread to other food producing animals. The HPA and VLA worked on the 2005 outbreak of *Salmonella* Java in cattle on a farm in the Midlands. It was possible that the exotic or ornamental fish were being used in drinking troughs to reduce algal contamination and therefore feasible that the Far East strain of *Salmonella* Java infected cattle through drinking water.

Peter Silley (MB Consult Ltd) asked whether the mapping around the SGI-1, particularly looking for virulence determinants, might provide some clues as to why these particular serotypes disappear over time. The origins of serotypes are known but the cause of their disappearance is unclear. John replied that the HPA carried out a study a few years ago with Bernard Rowe which investigated invasion potential of DT104 in *Salmonella* infections in humans in England and Wales. The organism did not appear more invasive in terms of human disease than other serotypes. In contrast studies in the USA have indicated that MR DT104 is highly virulent but this was not observed in UK infections. The Americans and Danes have published several papers on the 'enhanced virulence' of MR DT104. John thought that SGI-1 may contribute to virulence in terms of enhanced adhesion but further studies are required to investigate gene regulation within SGI-1.

Diane Newell asked how the information accumulated can be used to predict what organism might emerge next, in order to look out for it. John explained that new monophasic types of *Salmonella*, which are related to Typhimurium, are being observed particularly in countries in Southern Europe. In terms of drug resistance, multiresistant monophasic *Salmonella* organisms' strains are particularly common in Spain. John thought investigating the spread of SGI-1 from Typhimurium to other serotypes could provide an indication of what may be happening. In due course it is highly likely that the

island will become inserted into a plasmid, which will further enhance its ability to spread to potentially epidemic strains such as DT104.

SESSION IV: REVIEW OF THE FSA'S B14 ENTEROAGGREGATIVE *ESCHERICHIA COLI* RESEARCH

Project B14002: Detection of Enteroaggregative *Escherichia coli* in clinical specimens and foods

Dr Henrik Chart, Health Protection Agency

The contractor's B14002 project summary is included in Appendix 12.

The aim of the project was to develop a simple but sensitive test for the detection of enteroaggregative strains of *Escherichia coli* (EAggEC).

Genotyping tests for EAggEC were developed based on three genes *aat*, *aap* and *aaiA*, and a fourth *uidA* which could be used to confirm the presence of *E. coli*. The primers could be applied in real-time platforms and were able to detect EAggEC in pure culture, in foods with and without associated competitor organisms and in clinical samples.

The EAggEC were not found to have unusual growth patterns and a growth medium for detection was not identified. Although tests were developed to identify dispersin successfully a simple method for use in a routine laboratory was not identified. Spiking foods generally led to detection of the spiked organisms but naturally occurring components in minced beef was found to hinder the detection of EAggEC.

Overall the work has shown that there are few, if any, easily detectable phenotypic attributes that delineate strains of EAggEC from other strains of *E. coli* but that PCR primer sequences could readily detect EAggEC strains in clinical samples and foods, and could be used in real-time PCR and in microarray formats. As a result there is now a highly sensitive procedure for detecting EAggEC which can be set up and performed in any laboratory with basic molecular biology equipment and expertise.

Main discussion points

Mike Gasson asked how complete the correlation was for *aat* gene as there appeared to be very good correlation for *aat* and phenotype. Henrik Chart (Health Protection Agency) confirmed that tends to be the case. Mike asked whether this meant that there were no positive phenotypes without the *aat* gene. Henrik explained that this was not observed in the 'typical' strains whereas the atypical strains were very heterogeneous. In terms of typical strains, Mike asked whether they were 100% detected using *aat*. Henrik thought 100% detection rate for any test was probably asking too much, but the detection rate was very high. Mike asked if the primers being used in the PCR experiments were relevant, when extra genes, other than *aat*, were not being detected. Was there a possibility of sequence variation in the DNA sequences targeted by the primers causing false negatives? Henrik explained that it was an exclusion procedure so if the gene is not present there was no point searching any further.

Peter McClure asked whether the control strains used were from different pathotypes or were they commensal *E. coli*. Henrik thought the controls were almost certainly

different pathotypes because the HPA tends to have bacteria predominantly obtained from ill people. There are no doubt situations where commensal strains would be there but the vast majority would be pathogenic. Peter asked if the particular genotypic characteristics examined were unique to EAggEC. Henrik confirmed Peter's assumption.

Andrew Fox asked how big a problem EAggEC was in the UK. Henrik revealed that EAggEC is predominantly an issue in 3rd world countries and produces prolonged episodes of chronic diarrhoeal disease. There have been outbreaks of EAggEC in the UK and other parts of the world such as Japan. EAggEC is perceived not to be a major concern in the UK but this risk could be underestimated as they are difficult to identify and not readily looked for. Therefore the scale of EAggEC in the UK is not known. Andrew Fox then asked what would be the rationale for looking for EAggEC. Henrik claimed that there have been various foodborne outbreaks associated with EAggEC but was unable to provide any figures.

Eric Bolton pointed out that the results presented for milk indicated that the PCR for the *aap* and *aaiC* genes were positive but the *uidA* gene PCR was negative with 1 of the samples. This indicates that non *E. coli* Enterobacteriaceae share these genes and asked whether *aat* gene was detected in other Enterobacteriaceae. Henrik was unable to answer the question but thought that it was unlikely.

Ian Toth asked if the experiment on lettuce focussed on the leaves or the whole plant. Chris Baylis (Campden & Chorleywood Food Research Association) explained that bagged lettuce leaves were used in these experiments. Henrik added a lot of work was carried out to optimise the PCR method. Problems were encountered with starting the multiplex when using the *uidA* gene. This was eventually resolved but there was a huge amount of work looking at how these gene targets can be detected in multiplex as well as individually. With lettuce it would have been attachment of the organism to the leaves. Ian Toth explained that the roots are the nutrient rich part of the plant so pathogenic organisms are more likely to exist and colonise the plants roots. There would be some degree of superficial contamination of leaves, but the real contamination and infections are likely to occur on roots. Henrik mentioned that *E. coli* O157 has been shown to invade plants but the extent to which other bacteria become incorporated is unknown. Chris Baylis noted the points on internalisation and preferential binding particularly by *E. coli* O157 to root nodules and subsequent uptake, which had been the focus of some published research. Chris pointed out that study was interested in the potential for contamination of irrigation water, which can be applied to crops.

Project B14003: Development and validation of diagnostic tests for Enteroaggregative *Escherichia coli*

Dr Anna Snelling, University of Bradford

The contractor's B14003 project summary is included under Appendix 13.

Project B14003 aimed to identify genetic loci that are conserved among pathogenic Enteroaggregative *Escherichia coli* (EAggEC) isolates from food and clinical specimens. The project also aimed to use these loci to develop candidate DNA probes and polyclonal antibodies that would serve as the basis for molecular and non-molecular tests for the organism. If no marker was found that was unique to EAggEC then genetic

and phenotypic tests would be sought that might have the utility to identify a pathogenic sub-set. Finally the project also aimed to estimate the local prevalence of EAggEC among diarrhoeal cases in Northern England and build a collection of isolates not biased towards positivity with the existing CVD432 EAggEC-associated probes.

Using a collection of well characterized and phylogenetically sub-grouped EAggEC strains, two strategies were used to identify conserved EAggEC specific sequences. The first approach was to use repetitive element-based PCR-genomic profiling whilst the second involved screening hot-spots for genomic island insertion. The results showed that the EAggEC is genotypically heterogeneous and that no one marker could be developed as a tool for identifying all strains.

The data showed that EAggEC includes a diverse mix of strains with no features in common, other than the ability to show aggregation in the HEp-2 assay. Even for this feature, there are marked variations in the pattern seen. It is not possible to have a DNA-based test that would accurately identify all EAggEC, but the project has made progress with developing tests that pick out sub-groups of EAggEC, including those currently believed to cause more severe illness.

The finding of EAggEC in stool samples of 18.5% of patients with traveller's diarrhoea adds to the concept that EAggEC are important and widespread pathogens and hence further research into their properties is required. It was also determined that proposed surrogate tests such as the 'clump test' are too non-specific while a probe designed to identify diffusely-adherent *E. coli* recognizes a specific subset of EAggEC strains. Until clinical studies are able to delineate hypervirulent EAggEC-subgroups, EAggEC isolates should be identified by the HEp-2 assay, or where not possible, multiple genetic targets should be sought.

Main discussion points

Christine Dodd noted that open reading frames (ORFs) being evaluated as markers for EAggEC were found in the *E. coli* isolates from meat, and asked if any were *E. coli* O157? Anna Snelling (University of Bradford) explained that the collection of isolates were from the Sheffield laboratory and that they were unable place them into any of the pathogenic groupings. So none of these isolates were identified as *E. coli* O157 by conventional testing. Christine asked for more clarification on the meat isolates. Anna revealed that of the >400 isolates from meat and animal faecal samples, some were positive for these ORFs however none of them were positive for the CVD432 EAggEC-related marker. Christine noted that there were difficulties in expressing ORFs in *E. coli* when trying to make antibodies and asked if ORFs were expressed better in the EAggEC. Anna asked Jonathan Fletcher to explain this aspect of the work. Jonathan Fletcher explained that *orfZ0608* protein was detectable in cell lysates of the prototype strains O42 and was visible in membrane preparations. The protein is not expressed to the same extent in non-pathogenic strains carrying recombinant plasmids. Even under stringent control systems using IPTG induction, cell growth decreased as soon as protein expression started, particularly with Z0608, cell growth stopping completely. Christine asked if this was a problem of over-expression. Jonathan thought it was linked to the protein itself rather than over expression. Eventually, *E. coli* was left to grow virtually to the end of exponential phase before inducing the protein which is very transiently expressed. It was hoped that this approach would yield enough protein to be

used for immunisation work. However, the amount this method produced was only in the order of a few hundred micrograms.

Dominic Mellor expressed concerns about the predictive values of the tests because they would be affected by prevalence of the organism. Dominic asked how this information could be used and in what settings they are best applied. Anna commented the study tried to find a test that would encompass as many of the EAggEC as possible. However, this study has raised the question of which of the EAggEC **should** be detected and the lack of case control studies is a real problem in deciding this. IID1 has shown that normal healthy people are able to carry EAggEC but case control studies carried out in places such as Nigeria and Brazil disagree with each other as to which EAggEC subcategories should be targeted. Within the next few years, there will hopefully be more clinical data available that show whether these EAggEC with a particular set of genes are really important to investigate. Dominic Mellor added that a more specific case definition would be advantageous.

Henrik Chart observed that a lot of work was carried out on pellicle formation. If the calcium ions in the growth media were 'mopped up' the pellicle disappears, and thus the phenomenon could be related to charge. Henrik also pointed out that the "pattern X" adherence pattern described in the presentation looks similar to localised adhesion and asked whether localised adhesive strains were included as controls. Anna clarified that adhesion controls were used. Anna commented that "pattern X" was 2-dimensional. With localised adherence (carried out with 'HEp2 cells) the pattern is more 3-dimensional and usually dense. Whereas the "pattern X" adherence was in one plane and cells lined up very neatly within that area. Localised adherence-like strains were also examined and it was different to that pattern as well.

OPEN DISCUSSION SESSION INCLUDING FUTURE PRIORITIES IN THE AREA OF *CAMPYLOBACTER*, *SALMONELLA* AND ENTEROAGGREGATIVE *ESCHERICHIA COLI* RESEARCH

Chairman: Professor Bill Reilly

Bill Reilly reminded the audience that the purpose of this session was two fold. Firstly to determine how well the research presented on day 2 met the original research requirement (provided in the B14 foodborne diseases research review delegate packs) and secondly to identify what the research gaps are and what the future priorities should be.

Bill Reilly mentioned that other work streams are currently in progress that compliments the research being funded in the B14 programme and asked Jacqui McElhiney to provide a brief outline of *Campylobacter* research being funded by FSA Scotland. Jacqui McElhiney (FSA Scotland) explained that FSA Scotland is funding a research programme looking at the sources and geographical distribution of *Campylobacter* infections in Scotland. The work is being carried out by the University of Aberdeen, University of Glasgow and Health Protection Scotland. A large study, which is utilising MLST to type 97% of all clinical isolates (5,500) submitted in Scotland over a 15 month period, has recently been completed. Typing on a range of retail chicken isolates collected as part of the LACORS/HPA retail chicken survey has also been carried out. Additional sampling of retail chicken and liver samples from food outlets around North East Scotland and sampling of environmental faecal samples of cattle, sheep, pigs,

poultry, wild birds, companion animals and private water supplies were carried out. This gave an additional 1,100 food and environmental *Campylobacter* isolates. This work will be completed and published within the next few months. The MLST project will feed into another project which is looking at spatial and temporal differences in *Campylobacter* infection across Scotland and will use modelling techniques undertaken at Universities of Glasgow and Aberdeen. The project will also link MLST data with epidemiological data which has been gathered over the same period and involves cases from all of the Scottish health boards over that 15 month period as well as some historical data. Finally FSA Scotland is funding work to assess the role of private water supplies in *Campylobacter* infection in Scotland. This study was co-funded with Scottish Government after a sentinel surveillance study, which suggested a link between having a household private water supply and cases of human infection in Scotland. This is a significant programme of work and ties in well with the B14 *Campylobacter* research. Andrew Fox also added that the University of Liverpool are looking at 3,000 - 4,000 isolates from human infection as well as isolates from retail poultry. Defra have also funded a study with Oxford University to look at 3,000 isolates from human infection in the Oxfordshire area.

Bill Reilly invited delegates to comment on whether the projects presented on Day 2 had met the research requirements:

John Threlfall raised the point that strains of VTEC and *Salmonella* can be traced using PFGE through the food chain both nationally and internationally. This is not as yet the case for international investigations of *Campylobacter* infection. It is important to address whether the strains of *Campylobacter* found in travellers are different to the strains found in Great Britain. Diane Newell explained that no European groups are placing emphasis on MLST and very little in the USA. PFGE is considered to be the 'gold standard' across Europe so the UK stands alone in using the MLST approach for this organism.

Andrew Fox pointed out that in the USA, the CDC are to undertake a major MLST project similar to the research carried out in the UK. MLST is exclusively used in both Australia and New Zealand and is informing their food safety activity directly. Comparative data is available and at the last *Campylobacter*, *Helicobacter* and Related Organisms (CHRO) meeting, it was demonstrated that MLST is an international currency in terms of strain characterisation for *Campylobacter* and it is widely used in other parts of the world. Diane commented that at the last two CHRO meetings a statement has been made that the MLST data is extremely biased and there is a need to ensure samples are more representative.

Sarah O'Brien explained that soon to be published research from the UK, Denmark, New Zealand and Iceland suggests that poultry plays an important part in the human disease. Therefore should future research focus on carrying out intervention studies on the poultry supply chain?

Mike Gasson thought it was worth reflecting on the underlying basis of PFGE and MLST methods because they are complimentary rather than alternatives. PFGE provides a snapshot of the total genome based on its structure in its totality whilst MLST is sampling in precise way, particular regions of that genome. So mechanistically they are actually giving two sorts of insights into the organism. Leaving aside the practicalities of what works well in the time frame they are giving different windows of information which

are complimentary. They are not alternatives in a pure science sense but are actually additive.

Bill Reilly summarised that the aim is to try to identify a tool to help understand the epidemiology of *Campylobacter* and thus allow targeted interventions. Bill suggested that a panel of tools is required rather than one to achieve that end. Some of the work from the samples in Scotland may be an evaluation of how useful MLST will be in the real sense of providing information to public health for intervention.

In response to Diane Newell's earlier comment that some *Campylobacter* groups are clonal, Charles Penn emphasised that there are fundamental issues with the lack of clonality and potential recombinogenicity in some groups of strains. It should not be assumed that the epidemiological tools will be developed to trace strains internationally if the strains are constantly redesigning themselves. It is unclear how robust the majority of strains are in terms of having a durable molecular type.

Diane Newell agreed with Charles Penn. MLST is not appropriate for looking for *Campylobacter* around a farm because the technique has insufficient discriminatory power. A method like PFGE is needed and the disadvantages associated with it should be accepted. MLST should be used if comparing *Campylobacter* populations in humans and poultry. There is a tendency to put *Campylobacter* in the same 'box' as *Salmonella* and *E. coli*.

Bill then invited Eric Bolton to re-ask his question on *Listeria* from this morning to widen this slightly.

Eric Bolton thought the impact of interventions needs to be measured. This could be crude in terms of being semi-quantitative (for example, what's the number of case reduction) or much more sophisticated such as demonstrating that a particular strain had been removed from a specific part of the food chain by using an intervention. There is a need to think about the most appropriate typing method to do this and linking the intervention to providing that sort of evidence.

Eric Bolton commented that the UK has not followed the trend in the USA of developing a PulseNet equivalent for a wide range of food poisoning organisms in the UK. Is there a need for this to inform the FSA in a standardised way what's actually happening with these food poisoning organisms? John Threlfall fully agreed on this aspect and pointed out that electronic gel images can be transmitted around the world within minutes, yet it takes up to 10 days to do that in the UK. Bill Reilly asked if this should therefore be a recommendation for the FSA. Diane Newell added that MedVetNet worked hard with PulseNet Europe in developing the electronic systems to be comparable with CDC system of PulseNet and other international PulseNet equivalents. The IT barriers within organisations, such as the firewalls at the VLA and HPA, are preventing the efficient electronic swapping of data. Bill clarified with Eric that he was suggesting we need to use these technologies for looking at different organisms as well; and should not just concentrate on what is numerically the biggest organism at present. Eric Bolton agreed all the food poisoning organisms should be looked at.

Eric Bolton asked how important quantification of *Campylobacter* in foodstuffs is in relation to disease. Diane Newell responded that at the moment there it would make no difference if we knew as it is currently not known what the minimum dose is for human

infection, the variation in human susceptibility to these doses and which strains are more virulent or pathogenic than others. There is currently only 1 dose-response curve published for *Campylobacter*. This curve has been difficult to interpret but has so far been used in every risk assessment model.

Keith Gooderham (Private Veterinary Consultant) agreed with Diane and added that there is a need to know whether interventions are actually reducing the carriage of *Campylobacter* in poultry as opposed to eliminating it. Vaccines and bacteriophage interventions may, in the future, reduce the prevalence in poultry and could be useful in reducing the numbers of *Campylobacter* entering the processing plants. Dominic Mellor thought this was a very well made point because typically, the focus is on eliminating carriage of the organism, but actually, reducing the concentration is probably more achievable and may affect transmission dynamics and persistence considerably; this is also dependent on infectious dose, transmissibility and so on, in terms of understanding the dynamics of the infections within the host population. Diane Newell also added that there is a FSA B15 project being carried out, which is a critical review of interventions in poultry on farm and is due for reporting in autumn 2008. This study may give an indication of what interventions may be feasible.

Bill Reilly suggested that there is a lot going on in *Campylobacter* through different funding streams and there may be merit going back to the FSA and suggesting having a *Campylobacter* review covering the totality of *Campylobacter* research which should involve all funding groups.

Sarah O'Brien added that it is also worth remembering that ACMSF have recently written a fairly comprehensive report on *Campylobacter* in the UK and there are a number of research recommendations in that report that may be worth revisiting in terms of what has not yet been done.

Tom Humphrey asked what the current state of play is on method development. Is what we have heard about during the review the current state of play or has there been more recent developments that have not been covered under the review? Tom suggested that it seems likely that there will never be a method that can reliably detect EAggEC in foods. Gael O'Neill mentioned that the FSA had not funded any recent work in this area.

Henrik Chart suggested that there is a mixture of strains of EAggEC and the biggest hurdle to overcome is to do the testing as current testing is still quite specialised. The HPA carries out PCR on strains that arrive into the laboratory and gets an indication of what strains are actually out in the community. Henrik thought that making a detection test that works in a routine clinical food laboratory is a bit further down the line but a start has been made. Henrik thought that the potential chip technologies may provide more opportunities in the future.

Anna Snelling commented that it has been discovered that there is a polymorphism in the promoter of the human IL8 gene and people who have a particular form of that polymorphism are much more likely to develop diarrhoea due to EAggEC and get more severe inflammatory diarrhoea. There is a lack of studies looking at host factors rather than just looking at the properties of bacterial strains themselves. Anna made the point that in terms of test development and sorting out the epidemiology of infection and doing surveillance studies, if there are people in a population who are not susceptible

and people who are hyper-susceptible, that really does complicate matters. Anna thought that at present it was not feasible to send environmental health officers to collect DNA samples from the victim as well as the food sample, but perhaps would be in years to come. In future, looking at the DNA of the host may be just as important for sorting out a diagnosis as looking at the DNA of the organism, and that is really where micro-array/DNA chip technologies could come in.

Sarah O'Brien mentioned that there is a massive UK population based cohort study called UK Bio-bank which is attempting to get host DNA and a variety of other environmental risk factors on half a million people in the UK population. As far as Sarah was aware the study is not looking at infectious diseases, be they foodborne or any other type of infection, but this could be a huge resource that the FSA might want to tap into.

Referring to Henrik's presentation, Mike Gasson pointed out the *aat* gene correlates very well with the typical strains. If that is the case, there is a mechanism for detecting a subset of strains albeit recognising that the method is only picking up strains that have the *aat* gene. There is a possibility that the presence of that gene may not always correlate to a phenotype as there may be defective or unexpressed genes. It would not be surprising if an *aat* positive was seen that was negative for the phenotype test. The whole of this exercise is looking for a correlation e.g. looking for some genetic or protein factor that correlates to the phenotype. Mike thought that an easier approach to getting a test would be to genetically unpick the phenotype. Take 1 strain that is positive for the phenotype and then genetically understand how that phenotype is built up. This is similar to the difference between a) understanding the phenotype and then finding the test as distinct from b) correlating the phenotype with something that gives the test. Mike thought that the major obstacle is the heterogeneity. Mike explained that he would use one really typical strain and then understand the basis of this strains phenotype in genetic terms. This should hopefully give some pointers to develop a test. Henrik Chart commented that Anna Snelling mentioned in her talk that the 2 major strains of EAaggEC, 042 (which is in fact serotype 044:H18) and 172 have been sequenced. However these are not good model strains as they are quite unusual and this group of organisms as a whole are very heterogeneous. Therefore identification of one model strain is very difficult. If there is now an easier way to sequence many strains this should be done as sequencing one strain would not provide enough data.

Mike Gasson clarified that his point was not on sequencing but actually on genetically analysing a strain to understand the components that make up the phenotype. There could be a common phenotype spread around a multiplicity of variations but, at present, there is no understanding of which genes make up the phenotype being measured. There are some good correlations, for example, with the *aat* gene, but this may only give the phenotype in certain backgrounds and may need 6 other genes on the chromosome – genes which are currently not known. If this question is answered for one system using classical genetics to take apart the *aat* gene (and its genetic context), you will understand what the phenotype consists of. Henrik Chart made the point that there are certain situations where genes are switched on. Genes are inducible and of course that is a very important phenotypic test. However the right conditions have to be applied to know that the genes are present and switched on. Charles Penn said that if there is a typical strain that has not been previously sequenced, it would be quite simple to get it sequenced using modern sequencing technologies.

Anna Snelling mentioned that Jim Nataro's group in Maryland have been looking at the genetic make up of EAggEC for several years and it seems that in order to get the Enteroaggregative phenotype the key is the aggregative adherence fimbriae (aaf). There were 3 of these recognised and the problem was that this did not cover all the EAggEC strains, as there were strains seen that did not contain any of the 3. Nevertheless, Jim Nataro has just published work on a 4th type of adhesin which has addressed a lot of these blanks. Jim has now been able to account for the mechanism of the Enteroaggregative phenotype in quite a large proportion of strains. The next thing required is to see if the enteroaggregative phenotype actually correlates completely with being virulent.

Bill Reilly asked the delegates how much resource the FSA should continue to put into the area of EAggEC in terms of prioritising with other research demands. Diane Newell's impression was that EAggEC are a relatively small component of foodborne disease and that food is a vehicle for these bacteria rather than the source. In terms of priority, EAggEC must be relatively small compared to the huge number of other organisms.

Tom Humphrey thought that a major issue with defining research priorities is clarifying how the FSA undertakes research prioritisation. How much does the press, MPs and review processes such as the B14 review influence the Agency's decision making? Gael O'Neill explained that the Agency sees the review as a way of gathering information on what the scientific community thinks are important priorities, what research needs to be done and where it needs to go. This feeds into educating the Agency in terms of deciding which policies need to be implemented and how research is taken forward. There are other influences but the review is an information gathering exercise to finding out what the research community views are on FSA commissioned research in a specific area. There is a broader spread of people present at this review that might normally come into direct contact with the Agency and is an opportunity for delegates to share their opinions.

Tom Humphrey thought that the FSA has too many targets and target microorganisms and this is very influenced by Government, press and public opinion. This makes it very difficult for the Agency to achieve its targets. He suggested that the Agency should adopt a more focussed approach which only focuses on 2 or 3 specific targets such as reducing *Campylobacter*. Dominic Mellor fully endorsed Tom's suggestion, with the proviso of also considering the hosts' susceptibility. Dominic explained that single pathogen studies are useful to a point, but they do not explain the complete picture.

Sarah O'Brien pointed out that one thing that has not been mentioned regarding the host is the host's behaviour – and this needs to be investigated in a social science context. There is a tendency to focus on the behaviour of microorganisms but the behaviour of humans is usually ignored. The factors that might be common for a lot of these organisms, in terms of human behaviour, need to be investigated. Diane Newell agreed that social science should have an input, but also pointed out that recent international meetings and publications have provided quite clear guidelines about how disease and disease burdens should be prioritised. There are some good models (MedVetNet meetings and US prioritisation programmes) that the FSA could be using to prioritise research. Bill Reilly raised the slight concern that if you use a simple model there may be some organisms/disease that never appears above the priority threshold so there has to be some flexibility within that framework.

John Threlfall mentioned that carrying out sequencing of 'typical' strains can open up new areas to explore, such as virulence markers and antibiotic resistance genes and so should be carried out as a matter of urgency. In this respect, strains of *E. coli* and *Salmonella* that produce extended spectrum beta-lactamases are causing disease and deaths at the present time and particularly in relation to *E. coli*, the source of these organisms is not known.

Sarah O'Brien stated that future burden of illness studies need to be dynamic with the data being refreshed from time to time. When the interventions against *Campylobacter* and *Salmonella* are successful in the future, then the focus would shift to the lower priority organisms because their importance will increase once the prevalence of those that are important today has decreased.

Celia Caulcott suggested that the FSA is under pressure to do too much work and there is a tendency to follow external demands e.g. from politicians. However, there remain 2 or 3 absolutely key foodborne pathogens, notably *Campylobacter* and *Salmonella* where major issues are unresolved. The understanding of the difference of *Campylobacter* from other foodborne pathogens is increasing and Celia thought there was a need to understand the host-pathogen interactions, the physiology of the pathogens and what causes organisms to be pathogenic.

Bill Reilly asked how the collaboration of funding bodies works and whether there is a strategy set out for funding groups as a whole. Celia explained that the main funding bodies that fund research in the UK associated with microbiological safety meet about every 4 months. However, this is essentially in an information exchange context, rather than in terms of funding strategy. It would require a very high level decision by the 3 or 4 key players: FSA, Defra, BBSRC and perhaps MRC to actually recognise that there are some nationally important strategic objectives that need to be addressed to do with the health of the nation. The impact of climate change has not been mentioned thus far but its effect is going to be real because of the interaction of pathogens with the environment. Also the UK Clinical Research Collaboration (CRC) report on the research effort on various disease areas in the UK as against the impact on the economy needs to be revisited. The report shows there is inadequate research on gastrointestinal disease, so some big strategic issues need to be dealt with by the 4 main funding bodies.

Meirion Evans added that gathering information and quantifying long term sequelae becomes important if cases for research prioritisation are made.

From a farming point of view, Keith Gooderham suggested that to maintain pathogen free food producing animals, the animals are normally put into bio-secure units, but there is a now tendency to talk about a so-called "welfare friendly" approach of producing food from animals which would go against what has been discussed here in terms of food safety. Keith viewed this as being wrong. He felt that it's wrong to call extensive systems welfare friendly as they may not necessarily be and wrong to call intensive production welfare unfriendly as it's not necessarily welfare unfriendly. There are problems controlling diseases whether it be zoonoses like avian influenza or a foodborne pathogen such as *Campylobacter* where there may be higher levels in extensive than intensive farming. Use of land can also be considered; extensive systems use more land than they need to, they have lower feed efficiency and so on.

Bill Reilly cited this as a good illustration of the need to look at other funding bodies, as animal welfare and production systems and feeding is very much an issue for Defra.

Dominic Mellor added that we should consider whether if one pathogen is eliminated, a niche is created for some other bacteria and there has not been enough consideration of this. The current issue with *Clostridium difficile* seems to be a situation where, in striving to be microbe free, a niche has been created for something that is causing lot of problems. Celia Caulcott added that this is an area which might emphasise the need for social science and understanding what is going on in the home and also the catering industry.

Will Waites commented that Nottingham University had carried out two studies on behalf of Defra in the area of bacteria in plants. The findings showed that when growing sprouted beans and alfalfa and ordinary plants in a hydroponic system the bacteria actually get into the plants. Another problem with plants is that bits are broken off during washing and high levels of microorganisms can colonise these areas. It is difficult to remove the bacteria by washing so there is a whole area of concern with bagged salads. Nicola Holden (Scottish Crop Research Institute) added that this also continues the thread of looking at the whole ecology. Plant life is very complex and many enterics are already reported to be in association with plants. The organisms discussed during the B14 review are included within this group and it is very important to understand this phenomenon; both in terms of the basis of how the organisms colonise food plants in the context of the resident microflora, as well as their prevalence on fresh produce. Findings at SCRI concur with the Nottingham studies in that enteric bacteria firmly adhere to plant tissue and cannot be removed by simple washing. They have detected fluorescently-labelled bacteria within plant tissue (spinach roots), showing that they have a capacity to invade plants. The basis to invasion is unknown.

Ian Toth mentioned that genomics work carried out by the SCRI has found that *E. coli*, *Salmonella* and *Erwinia* are very similar and share a common ancestor. The only difference is in genomic islands that differentiate them in terms of pathogenicity and possibly other functions. Genomics work has shown that these islands in *Erwinia* determine its ability to grow, colonise plants and cause disease. SCRI have looked at the genomic islands in different types of *E. coli* and *Salmonella* and found that they contain genes that show considerable similarity to those found in genomic islands of plant pathogens. There is really no reason why pathogenic *E. coli* and *Salmonella* should not acquire genes required for life on plants given that they spend much of their time there. When SCRI started looking for collections of isolates from plants to study them more closely, and possibly sequence them, it was realised they just didn't exist. There are lots of clinical isolates but there is a real imbalance in isolates available from other sources, so more plant isolates are needed in order to better understand the life of human pathogens on plants. It is known that bacteria internalise into plants and they are important in disease yet the FSA and other organisations are doing virtually nothing in this area. Given that around 50% agricultural production is based on plants in the UK, animals and humans eat plants, animals also return waste to soil/plants, and the environment is as much plant and soil as it is water, it is a surprise that there is almost no work in that area. Bill agreed with Ian that this is a useful point and worth noting and perhaps the FSA should look at the role of plants in its set of priorities.

Anna Snelling thought that future research should focus more on prevention and the area of 'colonisation resistance' of our normal commensal gut flora. It is known that the

normal gut flora can prevent pathogens getting a foothold in that niche but it is not fully understood how this is achieved. Historically, this was something that was not explored in great detail because techniques were not there for monitoring the diversity of this enormously complex ecosystem. However, times have changed and the people interested in probiotics have done a lot to develop and apply these techniques. There is a real interest from clinicians as to how to protect patients when they are taken into hospital, by maintaining the natural gut flora, to stop them acquiring pathogens. There is also interest from the veterinary side, now that antibiotics are no longer used as growth promoters. Anna asked why the FSA are not doing more on looking at how the gut flora influences colonisation resistance and how this changes with age, because the gut flora in the over 60s is different when compared to younger populations. Bradford University have completed a study looking at MRSA carriage in the gut. Many clones now are enterotoxigenic and hence have the potential to cause diarrhoea and it can be detected in the faeces of about 5% of over 75s. Why do they succumb to carrying these strains compared to people with a 'younger' gut flora?

Tom Humphrey responded by mentioning the Bristol Veterinary Training and Research Initiative (VTRI) programme looking at commensal flora and immune development in pigs and chickens which was finding some interesting results. Tom mentioned the ACMSF Vulnerable groups working group is trying to address various issues including what makes older people more vulnerable to infection and looking at gut flora and immunity will be part of that. So Tom strongly reinforced the need for more research on host susceptibility.

Charles Penn advised that meaningful analysis of the gut flora issue is a huge challenge. Despite advances such as genome sequencing technology there is a lack of basic information on variation between individuals, what happens within individuals as time goes by or as they change their diets. There is a big move in the US to look at the human microbiome but such research on food animals is clearly lagging behind that. Charles thought that it would be beyond the resource of the FSA to tackle that alone, and it is not in a position to pursue this due to the lack of baseline information but hopefully that will change in the next few years. Christine Dodd suggested that the results from IID study may reinforce this because asymptomatic carriage and multiple infections in a single host are being seen. The interaction of the host with the pathogen is complex and something that needs further exploration.

Meirion Evans mentioned that some of the highest incidence of foodborne disease is in the very young and there are issues with immunity, for example breast feeding, and also risk factors for children. The case-control study in *Campylobacter* was done in adults, not infants or children and Meirion thought that this is an area that could be examined.

Diane Newell reminded delegates that there is a wealth of information on enteric pathogens such as *Salmonella*, *E. coli*, etc, however information on *Campylobacter's* ecology, physiology, pathology and pathogenesis is lacking. It is unclear how *Campylobacter* actually causes disease and Diane pleaded for more fundamental research that has an applied aim for this organism and this has to be done with joint research across the board (inter agency and academic).

Peter McClure highlighted that the FSA did a pilot study a few years ago looking at prioritisation of research across different themes. The outcome was not particularly successful but it was good learning exercise. Looking at prioritisation of research and

pathogens during the B14 review, there may be some lessons to learn in terms of things that should be focused on to help develop some sort of prioritisation tool to look at what's high, low and medium priority. Considering some of the things Diane Newell mentioned there are some elements of those to do with pathogens and food relatedness that would allow discrimination between certain pathogens. Three or four different factors may need to be chosen to allow development of that tool but Peter would endorse some time spent within the FSA looking at prioritisation and identifying some key characteristics that need to be worked on for the future.

Bill Reilly asked the delegates when considering the idea of inter-agency or inter-funding body co-operation, how the FSA could harness the work of the whole food industry (from production to consumption). Dominic Mellor responded that, in some sectors of the industry, there is a negative margin in some senses, such as farming, and that's a problem. If we wanted to do something about this we would need to empower farmers to take responsibility themselves. Legislation states that farmers should do this, but there is not a margin there for them to do so. Tom Humphrey concurred and commented that the UK farming industry is under huge economic pressure. Although supermarkets make a huge amount of money, very little of that is diverted towards research and it is very difficult to get them to share data because they regard these as confidential. Industry gets so overprotective that it is almost counterproductive.

Bill agreed there is a lot of information already held by industry but questions of commercial sensitivity, competition, freedom of information makes it very difficult to access some of the basic epidemiological information. Tom Humphrey suggested that one of the main problems is the "name and shame" policy of the FSA, especially with poultry companies who were made very anxious by the necessity for the FSA to make it clear who research was conducted with and what the results of the joint endeavour were. Bill pointed out that this is something that has been discussed recently within the FSA and there is a feeling that the FSA should perhaps be "naming and praising" those that do things well, rather than naming and shaming. Sarah O'Brien suggested that another thing this illustrates is that not only do the research community need to understand epidemiology of foodborne disease but also the epidemiology of food and this is not understood nearly as well as it should be in terms of risk based approaches to prevention.

The future research priorities identified during day 2's open discussion session were:

- Further interventions for poultry
- Quantification of *Campylobacter* in foods and on farms
- More refined tools for investigating *Campylobacter*
- Need for a 'PulseNet' equivalent in the UK for all foodborne pathogens
- Better understanding of genetic basis of EAggEC phenotype
- Screen Sera for antibodies to dispersin
- Host susceptibility and behaviour, which should involve social science
- ESBLs
- Role of plants in foodborne disease

SESSION V: REVIEW OF THE FSA'S B14 CLOSTRIDIA RESEARCH

Project B14006: Assessment of the ability of *Clostridium perfringens* strains to produce toxin after exposure to defined environmental conditions

Dr Kathie Grant, Health Protection Agency

The contractor's B14006 project summary is included under Appendix 16.

This project aimed to obtain data on a range of strains of *C. perfringens* in terms of their potential to produce enterotoxin and cause food poisoning. In addition, it was intended to establish a *C. perfringens* culture collection representative of different food poisoning outbreaks in the UK, while setting up a quantitative assay for *C. perfringens* enterotoxin and investigating sporulation methods. Further, jointly with IFR, to define heating regimes and the number of spores, vegetative cells and amount of enterotoxin produced, as well as validating data using beef slurry. Finally the study was intended to perform molecular typing on all *C. perfringens* strains before and after heating and cooling.

The results showed that the heat treatments used did not reproducibly increase toxin production. In food poisoning strains the enterotoxin (*cpe*) genes are usually located on the chromosome, whereas in the non-food poisoning strains *cpe* genes are located on plasmids. In general, strains of *C. perfringens* with plasmid encoded *cpe* genes have spores that are less resistant to heat. An important new finding from this study was food poisoning strains with plasmid encoded *cpe* genes.

Main discussion point

Mike Gasson asked whether a PCR based classification method was used to determine whether the location of the *cpe* genes was on the chromosome or on a plasmid in the strains. Kathie Grant (HPA) confirmed that PCR amplification was used. There are different insertion elements downstream of the enterotoxin gene depending upon whether it is chromosomally or plasmid encoded. Mike Gasson then questioned whether the robust conclusions highlighted in the presentation could be drawn using this method because essentially what was being tested for was the insertion element next to the enterotoxin gene and this did not necessarily provide direct evidence of the presence of the plasmid. Kathie explained that it was based on the evidence from studies, carried out by Bruce McClane's group, for establishing and evaluating the PCR assay. The PCR assay is now used by a number of different laboratories where they have used a large number of strains and the specific insertion elements have always been found to be associated with their respective chromosomal or plasmid location. Sequence analysis of *cpe* gene positive plasmids has shown that they are one of two types and the enterotoxin gene has not been found on any other plasmid in all of the studies carried out.

Christine Dodd was not sure why the project looked for the gene after heating and asked Kathie to provide explanation on what she thought was happening there. Kathie explained that there was a dogma that food poisoning is caused by strains with the *cpe* gene on the chromosome. Part of the study's aim was to see if heating caused the gene to be lost from either the chromosome or the plasmid or moved from one to the other because there is such a distinction in the populations between the plasmid encoded enterotoxin strains and the chromosomally encoded enterotoxin strains.

Tom Humphrey asked how the heating and cooling regimes used in the project were chosen. Kathie replied that the heating and cooling regimes were chosen in conjunction with Mike Peck at IFR and with Joy Gaze at Campden and Chorleywood Food Research Association carrying out the work. It was decided to use treatments that represented worst abuse and those that would be expected in domestic kitchens, catering and in food manufacturing. Tom Humphrey also asked what heating temperature is required to kill off the clostridia spores. Kathie mentioned that the majority of the spores will be killed off at a temperature of 95°C but the more heat resistant spores will remain. So very high temperatures are required which are not normally used and which would destroy the flavour of the food.

In relation to Tom Humphrey's question on heat resistance, Peter McClure (Unilever) suggested that it would be expected that some of the spores start dying off at 95°C particularly for heat sensitive spores. Peter asked whether the number of *C. perfringens* spores surviving after heating to 95°C was measured to determine if only a few spores in food could cause food poisoning. Kathie explained that this was not measured. What was found in a lot of cases was that the amount of toxin produced was related to strain variation and not to the number of *C. perfringens* spores in the food. Peter clarified that the key message from a food safety perspective is to prevent any number of *C. perfringens* spores, even if they are present in small numbers, from multiplying in foods. Kathie agreed but added that cooling foods quickly will prevent *C. perfringens* from multiplying.

Mike Peck (IFR) also commented that the heat resistance of spores is very high and heating at 95°C for 60 minutes is unlikely to reduce the number of heat resistant spores significantly. The literature suggests heating at 100°C for 200 minutes for heat resistant spores.

Project B14007: Food safety implications of potentially pathogenic clostridia
Dr Bernard Mackey, University of Reading

The contractor's B14007 project summary is included under Appendix 17.

The main aims of the project were directed at finding the proportion of *Clostridium butyricum* strains (isolated from natural sources) which carry the botulinum neurotoxin gene, as well as determining whether *C. tertium* and *C. bifermentans* produce toxins. In addition, the project also aimed to determine the temperatures, pH and solute concentration which would prevent the growth of *C. butyricum*, *C. tertium* and *C. barati*.

The toxigenic strains of *C. butyricum* do not appear to be commonly found in food but *C. butyricum*, *C. bifermentans*, *C. tertium* and *C. barati* can grow at pH values below those that prevent growth of proteolytic *C. botulinum*. The minimum growth temperature for toxigenic and other strains of *C. butyricum* was about 8°C. Where pH was a critical factor in food preservation, allowance must be made for the greater pH tolerance of *C. butyricum* compared with proteolytic *C. botulinum*. The pH levels which prevent butyric spoilage by *C. pasteurianum* should easily prevent growth of toxigenic *C. butyricum* strains in acid foods. However, spores of *C. butyricum* may sometimes be found in non-acid foods such as milk products and as a result, it is important to ensure that spores are eliminated from dairy-derived foods that will support their growth. From evidence

obtained from cell culture experiments (using Vero cells), it seems unlikely that growth of *C. bifementans* and *C. tertium* constitutes a significant risk factor in foodborne illness.

Main discussion points

Christine Dodd noticed that for the growth condition experiments a peptone extract was used and asked whether that was an animal based protein/peptone. Bernard Mackey (University of Reading) thought that the peptone extract used was animal based. Christine mentioned that a lot of these types of outbreaks have been associated with vegetables and asked whether a vegetable based medium was used and if a different range of conditions would be generated using this medium. Bernard thought that it could be possible that a vegetable based medium could contain something and would be worth exploring. A fairly rich medium was used during this project and should contain most of the nutrients, amino acids, etc required for growth.

Dominic Mellor probed for further detail on the sampling rationale used for looking for *C. butyricum* in soil and food. The table in the presentation makes interesting reading as there is varying sampling intensity and positivity. Dominic asked how the study team came up with the 978 samples was and whether this was predetermined by a power calculation to test a specific hypothesis. Bernard explained that they aimed for 1,000 samples. *C. butyricum* occurs in both dairy products and on vegetables, so the study team specifically looked at these types of food. Dominic asked where these food samples were sourced from. Bernard clarified that the food samples were taken from food retail outlets.

Charles Penn noted that the pH differences for the different clostridia were very small and asked whether buffers were used to make sure that the media would be stable under the experimental conditions and the pH would not be changing. Bernard confirmed that buffers were not used. Bernard explained that his former colleague, Richard Sherburn, used a batch of the same media. The pH was measured before and after autoclaving and adjusted. The pH of un-inoculated media was checked after 42 days and the pH was found to remain stable over this time. It was the ability to initiate growth at that particular pH that was looked for during the study.

Jean Banks (NIZO Food Research) mentioned that *C. butyricum* growth is a big problem in European cheeses and there have been a few sporadic cases in UK produced cheddar cheese. It is likely that these problems will increase with the move towards reducing salt content in cheese. Jean asked why cheese was not included in the list of samples. Bernard acknowledged that cheese was not included and it would have been interesting if this had been investigated.

Ian Toth mentioned that plants, like animals, have different defence capabilities and the type and age of a plant can effect whether microorganisms grow or not. He questioned whether any of these variables were taken into account when looking at tomatoes or the other plants? Bernard confirmed that a homogenate of tomatoes were used and that the variables mentioned were not considered during the plant testing.

Will Waites asked if *C. butyricum* in relation to silage was considered under this study. Silage is not used in New Zealand and there is no problem in dairy produce since clostridia were absent from the milk. Will asked if Bernard was aware of any other work

which has looked at clostridia in this area. Bernard was not aware of any research groups looking at silage but agreed it would be an interesting research area to investigate. Bernard mentioned a Polish study where soil samples were screened for botulinum type E toxin gene using PCR. The study found that *C. butyricum* was present in a wide range of environments and other vehicles.

Joy Gaze (Campden & Chorleywood Food Research Association) suggested that there is a lot of published work on anaerobic butyric anaerobes perhaps reducing the pH as they grow. In instances of botulinum outbreaks caused by these organisms she wondered whether Bernard thought that these organisms grew better in a reduced pH environment or whether the consequence of their growth could lower the pH of a product. Bernard didn't think they grew better in reduced pH environments but they do produce large amounts of acetic and butyric acid when they grow. *C. butyricum* growth only reduces the pH to about 4.0-4.5 but *C. pasteurianum* can take the pH down to 3.5. In some food commodities such as pickled olives, lactic acid bacteria are relied upon to reduce the pH quickly to prevent growth of the butyric spoilage flora.

Project B14008: Development and validation of a versatile model for predicting the growth of *Clostridium perfringens* during the cooling of meat

Dr Bernard Mackey, University of Reading

The contractor's B14008 project summary is included under Appendix 18.

This study aimed to develop and test an experimental system to validate growth of *C. perfringens* at all parts of a three dimensional food subjected to heating and cooling as well as incorporating *C. perfringens* growth parameters into the Fryer/Bellara model to produce a combined model for predicting growth in three dimensional solids. It also aimed for preliminary validation of growth in minced beef and solidified media under a range of dynamically changing heating and cooling conditions as well as to validate the growth of *C. perfringens* in a three dimensional solid under different heating/cooling conditions.

Model food systems were developed consisting of clostridial growth medium solidified with gelrite contained in cylindrical plastic 'sausages' casings or minced beef packed into casings of the same size. Gelrite was solid at temperatures above 100°C and allowed experiments to be carried out at temperatures above 100°C.

A computer code was written in MATLAB to solve engineering equations for predicting the dynamically changing temperature profiles within the cylinders during the heating. The resultant predictive model was tested experimentally with thermocouples placed in the gelrite or minced beef/beef sausages. Temperatures measured within the sausages at the centre or at different radii were very close to those predicted by the model under a range of different heating and cooling conditions. Growth of *C. perfringens* in liquid medium (Reinforced clostridial medium containing minced beef) was followed at a range of constant or changing temperatures and the viable counts compared with values predicted by the Baranyi model. The agreement between predicted growth and experimental results was very good. There were no major differences in the extent of the lag phase between spore and vegetative cell inocula under these conditions.

The Baranyi *C. perfringens* growth model was incorporated in the MATLAB temperature predictor model and simulation studies were carried out comparing growth at different

regions of a large cylindrical food product under different cooling regimes. Preliminary validation studies with the small sausages gave good agreement between model and experiment.

The significance of this project was the ability to calculate internal temperature and consequent microbial growth during cooling as a function of external temperatures and product size - this permits simulation exercises that will help to identify critical factors in risk assessment. The model can be extended to allow temperature profiles and microbial growth to be simulated in other shaped bodies using appropriate numerical methods. The results of this project will help to provide a powerful tool for evaluating the risk of *C. perfringens* growth under different heating and cooling conditions.

Main discussion points

Dominic Mellor asked Bernard Mackey to explain what the adjustment parameters were actually doing. Bernard explained that when a bacterial culture is taken and put into a new environment, there is a time period (lag phase) where the bacteria has to adjust to the new environment before they start to grow. So α factor is the readiness to grow. If an exponentially growing culture in broth was taken and subcultured it into a broth medium at the same temperature, the bacteria would start to grow straight away and α would be 1. On the other hand, a stationary phase culture or spores of *Clostridium* on food needs to undergo various changes before growth begins so there will be an adjustment function. The physiological basis of this with spores was not known, so by doing many experiments an empirical α value of 0.01, which allowed reasonable predictions of growth, was determined. Dominic inquired whether it was modelled as either a deterministic or a stochastic simulation, either 0.1 or 0.01. Mike Peck explained that α factor values range from 0 to 1 and is adjustable between these two figures. The value of 0.2 to 0.3 is more common for vegetative bacteria, whereas unheated spores will have a much lower value.

Will Waites mentioned that the old idea used to be that *C. sporogenes* gained a toxin gene and became *C. botulinum*. It appears that *C. baratii* has the potential to become *C. botulinum* as well. Will asked Bernard which clostridia species are likely to gain toxigenicity and become *C. botulinum*. Bernard acknowledged that this was a good question on lateral gene transfer which he did not have the answer to. It would be interesting to carry out a survey for neurotoxin genes, determine what range of bacteria contains the gene and potential for gene transfer between microorganisms. Mike Peck added that until 2 years ago it was thought that the neurotoxin gene in proteolytic and non-proteolytic *C. botulinum* was on the chromosome but recent evidence has shown it to be located on a plasmid, sometimes on the linear plasmid. So the implications for gene transfer are quite large.

Project B14009: Improved control of *Clostridium perfringens* including Project B13005: Expansion of the Perfringens Predictor model to include pH, nitrite and salt concentrations

Professor Mike Peck, Institute of Food Research

The contractor's B13005 and B14009 project summaries are included under appendices 10 and 19 respectively.

Clostridium perfringens is present in low numbers in many foods, especially meats and poultry, and is frequently associated with food poisoning when cooked foods are cooled too slowly. Project B14009 aimed to provide information on appropriate safe cooling regimes for meat and to provide a user-friendly computer software tool to predict growth of *C. perfringens* based on the temperature of bulk meats during cooling. The study produced a model, now freely available on the internet that predicts growth of *C. perfringens* during the cooling of meats taking account of sub-lethal damage received during heating and consequent lag, and agrees with the results from thirteen cooling profiles described in the scientific literature.

The Perfringens Predictor model, developed in project B14009, enables users to determine the growth of *Clostridium perfringens* during the cooling of meats but under optimal conditions of pH, 6-7, a low salt concentration and absence of sodium nitrite. The aim of B13005 (extension to B14009) was to develop the Predictor model further to enable the users to take account of different pH and sodium chloride concentrations and whether or not the meat is cured.

A newer more dynamic Perfringens Predictor model was produced that predicts growth of *C. perfringens*. This includes temperatures (15-22°C), pH (5.0-8.0), sodium concentration (0.5-4.0%) and sodium nitrite (0-150ppm) based on 84 growth curves from ComBase, the scientific literature and new in-house generated growth curves. The model was validated against a number of observations reported in the literature and in-house results on a number of different meats. An improved web-based version of the software called the Perfringens Predictor Web Edition is available at the ComBase website.

Main discussion points

Charles Penn asked Mike Peck whether there was much variation in the growth curves between the strains. If so, how was an average performance established? What was the range of naturally occurring strains i.e. would there be atypical strains that did not behave like the strains the model is based on. Mike Peck explained that the 20 strains used were involved in either food poisoning outbreaks or intestinal disease and were either provided by the HPA or obtained from National Collection of Type Cultures (NCTC). The heat resistance of the strains was assessed, as was growth following 3 different heating and cooling regimes. Approximately 8 strains were very heat sensitive and did not grow at all in the heating and cooling regimes, so these were eliminated very readily. Of the remaining 12 strains with spores which were heat resistant, about half showed good growth during the 3 heating and cooling regimes. It was decided to use the strains that showed good growth during the heat and cooling regimes and either a cocktail of 5 strains or the most robust strains were used.

Tom Humphrey asked Mike what he would say to the FSA and industry in terms of changing the current advice. In terms of the findings of the study, what would Mike suggest food caterers and processors do differently that they were not currently doing. Mike explained that the difficulty with the advice with *C. perfringens* is that one can either specify that heating and cooling should be done under certain conditions for example cooling from 95°C to 30°C in 2 hours and 30°C to 5°C in a further 2 hours. Alternatively the Perfringens Predictor model can be used. This is more flexible. The idea is that users could put their specific heat treatment and cooling profile into the model and ascertain whether it is safe or unsafe. So if everybody used the model, it

should eliminate the problems that might be encountered if people cool their meat too slowly. Also if there is a failure during the cooling process the model provides the basis for assessing whether that process is acceptable or not. For example if they are trying to cool over 5 hours and it actually took 6 hours the model provides a basis to decide whether they should continue with the food or dispose of it.

Tom Humphrey asked how this elegant science will be translated to normal caterers. If they visit the FSA website, what will they see now that they did not see 2 or 3 years ago? Madeleine Smith (University of Birmingham) suggested that the caterers would access this information through the Environmental Health Officers who can use the model. The Perfringens Predictor model has been very useful in assessing the critical limits in HACCP systems in small businesses and is useful in giving advice.

Peter McClure stated a lot of people in food industry are familiar in using models, are happy to visit websites and use them. However other people may not be as confident of doing this and may be apprehensive. Peter thought that there was an opportunity to use the model output to produce a simple 'look-up' table which provide some critical or maximum temperatures and times you must hold for. Peter asked whether Mike had received any feedback from the model users which suggested a simple 'look-up' table as another output. Mike mentioned that he had not received any feedback for a 'look-up' table. The development of this model is an attempt to move away from being prescriptive in this way. A couple of food companies did provide examples of heating and cooling profiles that were used as part of this study and these were shown to be very safe. Bernard Mackey suggested that it would be possible to put some of Phil Robbin's data on the effect of size on cooling into a 'look-up' table. If you have a certain size of meat joint you could roughly predict what the temperature might be in the centre and combine this with the Perfringens Predictor to give some advice. Mike also added that in the past the FSA and other regulatory bodies have provided advice on what is acceptable and lists are available from the USDA in America.

Bernard pointed out that there is a trend for celebrity chefs on television to cook at very low temperatures close to 50°C for several hours. It is well known that the maximum temperature for growth of *C. perfringens* is between 40°C to 50°C and it grows rapidly at 48°C doubling every 12 minutes. The risk with *C. perfringens* is if a large number of cells are consumed, they sporulate inside the gut and produce enterotoxin. Bernard went on to ask Mike considering *C. perfringens* can sporulate at 48°C; would it sporulate in food such as large meat joints and form enterotoxin because if it did not there is a reduced risk but if it does then there is a real risk. Mike confirmed that *C. perfringens* would grow very quickly if it is held at 48°C for a prolonged period of time but was not sure whether it would sporulate on the food. Although *C. perfringens* strains sporulate readily in the intestines, it is difficult to get strains to sporulate on food and culture media.

Bill Reilly asked, at an environmental health level, whether the model was being used as a tool for caterers rather than food manufacturers. Mike Peck added that quite a few Environment Health Officers and Caterers have attended IFRs ComBase course of which between 100 to 200 people attend each year. Madeleine Smith thought that the model is not being fully utilised as well as it could be at the small and medium size enterprises level. Part of the problem is the resistance in the enforcement sector with using any time temperature cooling regimes that exceed 90 minutes. This comes from the Department of Health's chill guidelines which are strictly adhered to by the

enforcement community. There is an issue of getting this model, its advantages and limitations to enforcement. The model is extremely useful for all types of food businesses, particularly for caterers and small manufacturers to be able to validate their critical limits. But there is an issue of explaining the model to the enforcement officers as they would be the ones to use it and accept those limits from caterers as meeting the legal requirements. Bill suggested that some feedback to the FSA would be to see how the Perfringens Predictor model fitted in with other chilling guidance.

Tom Humphrey asked what the most common food vehicles for *C. perfringens* are. Mike clarified that red meat is the typical vehicle but *C. perfringens* has also been associated with stewed dishes that have been held at the wrong temperature. For example in the USA there had been a large outbreak of *C. perfringens* associated with stewed cabbage. Kathie Grant also added that *C. perfringens* has been linked with Shepherd's pie and chicken. There has also been an increase in outbreaks associated with catering at Indian weddings where *C. perfringens* has been found in dhal as well as in chicken curry.

Tom Humphrey suggested that normal cooking does not kill off the spores and the rate of cooling is the preventative step in reducing *C. perfringens* associated food poisoning. Mike agreed and also stated that the D values could be around 200 minutes at 95°C so by subjecting canned food to the botulinum cook spores will be eliminated but normal cooking would not have this effect. So it is more of the case of cooling food rapidly and sufficiently.

Tom Humphrey asked how common clostridia were on raw beef for example. Mike said that spores of *C. perfringens* are very wide spread whereas spores of *C. botulinum* spores are generally present in low numbers.

Will Waites commented that *C. perfringens* are the most common spore forming organism in the environment and that it can be isolated from soil, rivers, food, etc. Studies have shown that not many *C. perfringens* are present on doner kebabs but these can multiply to large numbers if held at the incorrect temperature. Can the model deal with food like kebabs? Mike further commented that in principle the answer was yes, although he was unsure of what heat treatment or what temperature the kebabs are held at.

SESSION V: FUTURE RESEARCH PRIORITIES

Chairman: Professor Bill Reilly

Bill Reilly asked the audience to comment on whether they thought that the clostridia research presented on day 3 addressed the research requirements and whether there were any research gaps that the FSA need to consider in the future.

Charles Penn highlighted that one of the questions put to Kathie Grant earlier was why she thought that heat treatment affects toxin production and the answer was that the research call asked for it. Charles thought it was slightly odd that there did not seem to be a good basis for thinking that there would be a clear influence of heat on the actual toxin formation. That particular sentence in the research call seemed strange. Mike Peck clarified that when the call was published there had been a couple of weak

publications which indicated that this might be the case but on reading they did not seem to be convincing.

Christine Dodd thought that it was good to see projects like B14008 and B14009/B13005 produce very useful and tangible outputs. The Perfringens Predictor is able to predict the growth of *C. perfringens* during cooling whilst the B14008 model is able to predict temperature relationships in 3-dimensional shapes and can be extended to other shapes or sizes. Both these models should be integrated as a facility alongside ComBase so that users could look at the temperature cooling profile their food products are generating and calculate the growth of *C. perfringens*. She was unsure what the future plans are for utilising these predictive models. Charles Penn agreed that inserting the shape and size parameters into the Perfringens model would produce a more comprehensive model.

Phil Robbins (University of Birmingham) explained that it is possible, with the software tools available, to model temperature profile accurately in any 3-dimensional shape imaginable with varying materials e.g. a large meat joint containing a bone in the centre. Simple cylindrical shapes were chosen for the project B14008 to allow development of a simpler mathematical tool for predicting time-temperature profiles and make it easier to measure and carry out the microbiology. It is possible to generate complex tools that allow users to enter more specific parameters such as type of meat, shape and diameter of meat and the heating and cooling profiles the meat is exposed to. The model would then provide an estimated time-temperature profile for that meat at different radii. This data could then be combined with the Perfringens Predictor model to get a prediction of the growth of *C. perfringens*. However the modelling of shapes can not be easily added to a web browser as it requires specialist software and expertise. It is possible to model simple shapes using software which could be developed for a web interface or excel. These shapes could then represent certain foods for example a 'slab' could be applied to a sandwich, a cylinder to sausages or a sphere for chicken.

Will Waites commented that the variation of spores in terms of heat resistance poses an interesting question as there is a general theory that more heat resistant spores are produced by bacteria growing at higher temperature. However *C. perfringens* goes against this logic as it produces both heat resistant and heat sensitive spores. More research is needed to determine the fundamental importance of heat resistance in spores and to understand these differences, especially the heat and pressure resistance of spores grown under different conditions. Bernard Mackey emphasised that very little research over the last 15 years has focussed on spore-forming organisms, which are very important to food safety. Further research on the fundamental aspects of spores would compliment the work that has been carried out under the B14 programme.

Mike Peck said that there are many orders of magnitude of heat resistance for spores of *C. perfringens* and this is unusual for a bacterium. In terms of a risk assessment it is important to understand why this is the case. Bill Reilly asked whether this would have a practical implication on how the food industry would manage the risk; do they not always assume that they dealing with the worst case scenario? Mike agreed but stated that if the heat sensitive spores are 100 times more common than the heat resistant spores then this would have a big impact on risk assessment. Bill summarised that the

distribution of heat resistant and heat sensitive *C. perfringens* in the wider environment and the problems associated with cross contamination is unknown.

Peter McClure also claimed that industry would be interested in using information on the load of heat resistant spores on food to target their heat processes. For example if a food company knew a specific raw material contained heat resistant spores, then the heating-cooling processes used could be redesigned to cater for this. However a tool is required that allows the ability to measure the levels of heat resistant spores. The tool should identify specific characteristics that provide an indication of heat resistant spores in the food. It also needs analytical capabilities that can be linked to the design of the process.

Mike Gasson commented that the question on the evolution of clostridia strains and the horizontal transfer of neurotoxin genes is unanswered. This still remains an important research area to address and an approach should be used where neurotoxin genes are looked for first and then it is determined where these genes are from.

Bernard Mackey agreed with Mike's statement about looking for the toxin genes and the organisms that carry them. Research carried out in China has revealed *C. butyricum* toxigenic types are very common in the soil. A survey undertaken in Argentina for toxin (by animal testing) did not find *C. butyricum*. It would be interesting to know about the distribution of organisms that might carry the toxin genes. When the importance of *C. perfringens* was first recognised, there was a feeling that the strains that did not produce heat resistant spores were more frequently associated with foodborne illness. This is no longer thought to be true but it is unclear with all the new strains that have become available whether there had been any examinations of the heat resistant spores and the amount of toxin they produce.

Kathie Grant explained that the production of toxin is going to be difficult to determine using *in vitro* methods because the levels depend on the condition in the gut and small intestines which varies depending on the people. From her experience of dealing with outbreaks, Kathie suggested that there is a tendency to find large groups of people that have consumed food but not all will get the symptoms of *C. perfringens* food poisoning, even though they ate the common food that has been implicated. This maybe a dose-dependant phenomenon where some individuals may have eaten an area of the food that did not have a large number of spores.

Mike Peck commented that 30 years ago there were only 3 clostridia known to produce botulinum neurotoxin there are now 6 groups. It has recently been demonstrated that the neurotoxin gene is located on a plasmid for many strains and that this was not previously known. There are bacteria in the environment that are capable of obtaining pieces of DNA by horizontal gene transfer, for example *S. aureus* and *C. difficile*. This is implicated in the problems associated with the hospital environment and antibiotic resistance genes. There is also information available that suggests some bacteria may be able to acquire *C. botulinum* DNA which is worrying. Typing methods are being developed that allow investigations of clusters of clostridia associated outbreaks. A study in California looking at infant botulism found that different clades or small groups of strains emerge and disappear with time and location. It would be useful to carry out research looking at the horizontal transfer of neurotoxin genes and its spread amongst different strains of bacteria.

Charles Penn mentioned that years ago when 16S sequencing first started, clostridia were shown to be phylogenetically very diverse and asked whether this is still the case? How close is *C. butyricum* to *C. perfringens* phylogenetically and what is its nearest neighbour? Mike Peck replied that when Dave Collins looked at 16S ribosomal RNA of foodborne proteolytic and non-proteolytic *C. botulinum* (Groups I and II), they were more different than *B. cereus* and *S. aureus*, so they are extremely diverse. IFR has developed a microarray for proteolytic *C. botulinum*. When using this microarray to compare very different species of clostridia it was found that they were too divergent to give any meaningful results. For example the results obtained for non-proteolytic *C. botulinum* and *C. difficile* were similar to those for *Campylobacter*. Charles said that trying to predict the horizontal transfer of these neurotoxin genes involves looking at very rare events. So there does not seem to be a framework to try to rationalise that at the moment. Mike Peck was unsure if the transfer of neurotoxin genes were rare events but stated that they do occur.

Christine Dodd wondered if there had been research on the genetics of heat sensitive and resistant *C. perfringens* and whether there are variations in Small Acid-Soluble Spore proteins (SASP). Mike Peck explained that 3 genomes have been sequenced and 70% were identical and 30% variable, and that a more recent study had highlighted the importance of differences in SASP proteins.

Madeleine Smith suggested that the FSA should consider producing a model similar to the Perfringens Predictor for *B. cereus* in boiled rice. Bill Reilly supported that view that if a model has been shown to be successful then the FSA should consider using the model for other conditions and foods.

Mike Peck explained that in Canada last year, three outbreaks of adult infectious *botulinum* were reported. These outbreaks were very rare and were associated with patients with underlying conditions, and that one had ingested the spores of proteolytic *C. botulinum* from peanut butter. The heat treatment given to peanut butter is not sufficient to kill the *botulinum* spores. The FSA may wish to speak to John Austin at Health Canada on whether advice is required for patients with underlying conditions (e.g. Crohn's disease patients).

Will Waites asked if *C. difficile* is being looked at in foods. Peter McClure revealed that there are a couple of reports that reported *C. difficile* in foods back in the 60's and 70's and offered to forward the reference to anyone interested.

Gaps in clostridia research and future priorities for the FSA

The future *Clostridium* research priorities identified during day 3 open discussion session were:

- Integration of the B14008 shape/size model into the Perfringens Predictor growth model. This involves expanding the B14007 model to consider other simple shapes and possibly have a web based tool.
- More fundamental work on heat resistance of spores including the distribution and load of heat resistance and heat sensitive spores in the environment.
- Evolution of clostridia strains and the lateral transfer of toxigenic genes (Plasmid borne toxin genes)
- Adult infectious botulism and the association with peanut butter, heat processing issues and advice for people with Crohn's disease.

- Production of a predictive model for *B. cereus* and other *Bacillus* spp. in rice

Bill Reilly asked the audience to now consider the whole B14 research programme - did the B14 research projects address the objectives of the B14 research programme (ROAME A document) and did they provide good value for money?

Mike Gasson suggested that a better understanding of *Campylobacter* is still required and it is difficult to type. Sarah O'Brien suggested the routinely typing of all *Campylobacter* isolates should stop as it does not appear to give any public health benefits. Mike Gasson's opinion was that an effective typing method that allowed identification/discrimination of *Campylobacter* strains is needed no matter how difficult it is.

Tom Humphrey mentioned that typing has become the end point rather than a tool. There are current concerns that chicken causes, 40%, 50% or 60% of the half million cases of *Campylobacter* infection per year. Tom felt that part of the problem with the FSA was that they were influenced by other agencies/bodies and asked to look at a whole range of microorganisms. The FSA effort is too diluted because of that necessity and they are unable to focus on a specific problem. The typing of *Campylobacter* has not told us anything that we do not already know and one of the outputs of the ACMSF working group was to stop typing *Campylobacter*. The number of *Campylobacter* cases has not got measurably worse since they stopped typing in Scotland. The key issues should be how *Campylobacter* gets into food and how it survives.

Bill Reilly stated that routinely typing clinical isolates to help identify a source and intervene successfully has been shown to be totally ineffective. However if we want to better understand where to intervene in the future, a typing scheme is needed to show the intervention point.

Celia Caulcott commented that the biological meaning of *Campylobacter* typing in terms of epidemiology, the host and pathogen behaviour is missing and need to be resolved. There is a lack of understanding of physiology, pathogenicity and host (human, chicken, cattle) interactions of *Campylobacter*. Celia also suggested that the FSA should be considering whether the relative incidence of *C. perfringens* infections and *Campylobacter* infections should be reflected in the levels of funding, with the implication that funding for *C. perfringens* would be reduced. Mike Peck strongly disagreed with Celia. The estimates from epidemiologists from the HPA have ascertained that *C. perfringens* causes more deaths than *Campylobacter* in the UK and is also second to *Campylobacter* in terms of the number of cases. So *C. perfringens* is important and warrants further investigations.

Peter McClure suggested that in terms of the main objectives of understanding *Campylobacter* better, the FSA is better placed working closely with agencies like the BBSRC in terms of generating research projects that improve this understanding. With the limited resources available to the FSA the basic background research on *Campylobacter* and other microorganisms should lie with funders like the BBSRC and closer collaboration need to be put in place.

Tom Humphrey raised a point on how the FSA uses the information that their research projects produce. Chlorinating drinking waters, changing farmer's footwear and adopting other hygiene interventions on poultry farms will actually reduce the incidence

of *Campylobacter* in those animals. However, it is slightly unclear how you would use the very elegant science on clostridia to change the advice on the web. The difficulty is that the way some projects are conducted does not necessarily give you the information that can be used in an easy manner. Mike Peck agreed with Tom on the need for advice that can be realistically used. The advantage of the Perfringens model is that it gives industry and EHOs the framework to determine if the heat or cooling treatment used is satisfactory or not. It also provides a decision on whether a meat can be used or discarded if the heating/cooling profile has failed, which in turn has important financial implications for them.

In response to Tom's comment on how the FSA uses its research, Bill replied that it is important to recognise that the review is only considering the B14 research programme with its specific set of objectives. There are a further 8-9 research programmes, 1 of which is specifically targeted to risk management and the review discussions might feedback into the other programmes as well. It may be worth considering the emphasis that goes between the research programmes for example taking research from B14 and putting more emphasis in the B15 poultry programme.

Peter McClure raised concerns about not looking for ways to optimise transferring outputs of this type of research into practice. For example the teaching sessions at IFR on Perfringens Predictor/Combase are excellent and a good group of people attend these but how can it be ensured that the majority of the food handlers in the UK are aware of those tools and use them? Also how can behaviour in food management and manufacturing be changed? This is a key aspect that the FSA needs to understand. A paper produced in early 2008 by a team led by Cardiff University (Sarah O'Brien was a co-author) showed that HACCP had no impact on the occurrence of outbreaks of foodborne disease when the management systems of premises that had experienced a foodborne disease outbreak were compared with control premises. The fact that they had recently been trained in HACCP had no impact at all. So the translation of the outputs of research programmes like B14 into manageable messages and getting people to change behaviour is key.

Mike Peck raised a comment that could be applied to all research programmes. There is a tendency with the FSA and other bodies for papers and outputs to be published after the research has finished. Mike suggested that the FSA should ask contractors to publish outputs/findings during the projects timeframe. The FSA should also place emphasis on research that has impact (such as publications or holding meeting) and make it a crucial part of the study. Bill agreed and clarified that this is an area the FSA is looking at. There is difficulty in putting information in the public domain before the research has finished because many of the scientific journals will not publish. There is a specific group in the FSA looking at placing information in the public domain as soon as possible.

Charles Penn noted that the basic understanding of *Campylobacter* is essential but it is not the remit of the FSA. There have already been some useful on-going BBSRC funded research programmes such as oxidative stress survival in *Campylobacter*. Some of those research programmes could lay the foundations for subsequent applications or interventions that the FSA could apply. There needs to be more knowledge of what is happening at the basic research level and updating and exploring these opportunities as they arise to then develop interventions.

Christine Dodd thought that in hindsight there were a few projects in the B14 programme that did not progress very well and may be due to the lack of basic understanding of the organisms which led to obstacles in carrying out the work and producing the expected practical outcomes. In the future the FSA should ensure that enough basic science has been established before commissioning applied research.

Ian Toth noted how very little genomics work has been undertaken, whether it is the typing and understanding of the biology of organisms or looking at the evolution and horizontal gene transfer. Genomics is an area which could be better exploited by funding agencies. Five years ago a genome sequence was done on a member of the enterobacteriaceae for £200,000. Next year you could possibly do 100 genome sequences on *Campylobacter* for the same cost. This would produce huge amounts of information on typing, ecology and gene transfer. Charles Penn agreed, however he also suggested that it was more for BBSRC to fund. In 1-2 years time the FSA should see what they can get out of genomics in terms of underlying biological knowledge and build on the foundations.

Dominic Mellor made the point that in terms of improving surveillance and epidemiology, the FSA needs to clear about what the priorities are. For example, when considering *Campylobacter*, if the FSA interests is purely foodborne disease the surveillance of all *Campylobacter* is possibly not relevant. In terms of *Campylobacter* disease attribution, how much is attributed to food and how much is attributed indirectly to environmental contamination which subsequently gets onto food. Dominic also stated that the variation of geographical rates of disease was not well addressed by the research and questioned why this was investigated in the first place. If the objective was to implement effective interventions, the FSA could usefully adopt a more ecological approach to understand where surveillance should be targeted and to understand the hosts' and organisms' biology. The FSA also need to determine whether surveillance should be carried out nationally or on a small scale.

Bill Reilly asked the B14 delegates to consider all the research gaps identified during the review and to suggest whether any should have a higher priority.

Charles Penn felt that bacterial physiology, such as how foodborne pathogens survive in the environment, has not been adequately covered by the B14 research programme and therefore should be a high priority. Anna Snelling agreed that none of the B14 research projects have covered the physiology and behaviour of organisms outside of the host and food matrix and how these organisms interact with the physical environment.

Tom Humphrey explained that one of the issues that were discussed by the ACMSF is funding gaps. There is a situation where if the FSA will not look at the survival of particular pathogens under certain conditions, it is also not perceived to be attractive enough for the BBSRC to look at either. With the exception of the work at University of Sheffield, and some fine work on oxidative stress, there is almost nothing funded by the BBSRC on the survival of pathogenic microorganisms. Due to policy reasons the FSA and Defra do not want to fund work on survival profiles and how survival is influenced by the environment. This is a gap that could be addressed through joined up thinking between FSA, Defra and BBSRC as there is a lot of fundamental work being funded that is not resulting in interventions. Bernard agreed that there was a 'no mans land' between the fundamental (BBSRC) and practical (FSA and Defra) research.

Will Waites mentioned that *L. monocytogenes* is found in the drains and blast chillers of food production plants whereas *Campylobacter* can not be isolated from similar places as they tend to die off. There are enormous differences between different strains of *Campylobacter*. He questioned whether people were working with the right strains or if there was a need to focus on the more resistant strains. Are consortia of microorganisms (for example, *Pseudomonas* and *Bacillus*, *Acinetobacter* and *Listeria monocytogenes* present in food processing environments), perhaps attached to surfaces in a similar way to which bacteria find suitable environments in the buccal cavity.

Bill Reilly asked the audience whether more emphasis should be placed on *L. monocytogenes* as this has not been picked up as a priority during the review discussions. Kathie Grant suggested that *L. monocytogenes* should be a priority research area.

Christine Dodd stated that host susceptibility, host-pathogen interaction and factors within the host that influence this interaction are the key issues that should be addressed by the FSA.

Bernard Mackey commented that if you look at how to strategically reduce foodborne illness there are 4 main areas that should be targeted. These are carriage of microorganisms in the host, hygiene in slaughterhouse/harvesting, food processing and interventions in the home. The extent to which interventions at each of these stages is useful depends on the physiology of the organisms. There are gaps on *Campylobacter* and *E. coli* carriage in live animals – for example why they can be carried by these animals with no symptoms but when they get into humans they cause illness? Very high numbers of *Campylobacter* are carried in the caecum but it is not known why. It is difficult to reduce *Campylobacter* numbers in the caecum as they are so well adapted to that particular niche. *Campylobacter* has a very small genome and very few stress responses but somehow manages to survive in the environment whereas *Salmonella* has a vast battery of interconnecting stress responses. What is the difference between these 2 organisms in terms of how their stress responses allow them to survive or not? Together the BBSRC and FSA/Defra should focus on the physiology and feed this into the broad strategy of where to intervene and what is appropriate for different organisms.

Research priorities identified during the B14 Foodborne Disease Research Programme Review

The main research priority areas identified by the B14 review delegates were:

- Cost effective study taking into account of chronic sequelae and acute infection
- Host susceptibility and behaviour of pathogens such as *Campylobacter* and *L. monocytogenes*
- Understanding the basic biology of *Campylobacter*
- More refined tools for detecting *Campylobacter*
- Need for a 'PulseNet' equivalent in the UK for all foodborne pathogens
- Evolution of Clostridia strains and the lateral transfer of toxigenic genes
- More fundamental work on the distribution of heat resistant and sensitive spores in the environment
- Physiology and behaviour of foodborne pathogens

Further comments to be fed back to the FSA

Other general comments on research that should be fed back to the FSA were:

- Adopt a more strategic approach which concentrates on fewer targets (reducing *Campylobacter*) and investigate these targets in more depth
- Future research should consider ecological aspects such as the changing environment and microbial communities
- Ensure that adequate basic science research is carried out before commissioning applied work
- Closer collaboration between the FSA, Defra and BBSRC to address the gap between the fundamental and more applied research
- The Agency is failing to attract a wide range of contractors such as Universities to carry out its research
- Earlier dissemination of research

5. CLOSED REVIEW

In order to help carry out the research evaluation process the Agency also referred the research projects to an external panel of experts who, in a private meeting following the open review meeting, evaluated each reviewed project⁶ for scientific quality, policy relevance and value for money. The panel scored each of the projects and the B14 programme for scientific quality and policy relevance following discussions to reach a consensus view.

Individual contractors were given anonymous feedback on the evaluation of their research projects by the Expert Panel so that both the Agency and its contractors benefit from the review process.

The closed review session was chaired by Professor Bill Reilly and the Expert Panel Members consisted of Professor Tom Humphrey (University of Bristol), Professor Christine Dodd (University of Nottingham), Professor Mike Gasson (Institute of Food Research), Professor Charles Penn (University of Birmingham), Dr John Cowden⁷ (Health Protection Scotland) and Dr Dominic Mellor (University of Glasgow).

Agency officials and the B14 Foodborne Diseases Research Programme Advisor, Professor Will Waites (University of Nottingham), attended as observers and to take a note of this meeting but had no involvement in the proceedings.

⁶ Please note that Projects B18021 and B14009/B13005 were not evaluated in the B14 Programme Review closed session. Project B18021 is an on-going study whilst B14009/B13005 was previously evaluated under the FSA B12 Microbiological Risk Assessment Research Programme Review, which was held in March 2007. Further information on the B12 MRA Research Programme Review, including the proceedings are available at: <http://www.food.gov.uk/science/research/researchinfo/foodborneillness>

⁷ Dr John Cowden attended Day 1 and 2 closed review sessions via telephone conferencing

6. CONCLUSIONS

Following an assessment and detailed consideration of the discussions and recommendations made during this review the Agency will determine if there is a need to continue with or modify the foodborne diseases research programme.

At this time, the Agency will consider consulting with interested parties to develop any future research priorities for foodborne diseases research. The views from the delegates, attending the B14 review, will be considered together with the reports.

The proceedings of this review meeting will be published on the Agency's website in due course.

APPENDIX 1: B14 FOODBORNE DISEASES RESEARCH PROGRAMME REVIEW AGENDA

www.food.gov.uk



AGENDA

REVIEW OF THE B14 FOODBORNE DISEASES RESEARCH PROGRAMME

23rd – 25th June 2008
Training and Conference Centre, Radcliffe House,
University of Warwick, Coventry

Day 1: 23rd June 2008
Lecture Room 4

09:30 – 10:30 **Registration with morning tea and coffee**
Lounge

SESSION I: INTRODUCTION

10:30 – 10:40 **Chair's opening remarks**
Professor Bill Reilly

10:40 – 10:50 **Background to the Food Standards Agency's B14 foodborne diseases research programme**
Ms Gael O'Neill, Food Standards Agency

SESSION II: REVIEW OF THE FSA'S B14 EPIDEMIOLOGY OF FOODBORNE DISEASES RESEARCH

11:00 – 11:15 **Project B08006: Ascertainment and enhancement of gastrointestinal infection surveillance and statistics (AEGISS)**
Dr Peter Hawtin, Health Protection Agency – Southampton General Hospital

11:25 – 11:45 **Project B14001: Food poisoning and foreign travel – a study of population health burden and risk factors for antibiotic resistance**
Dr Meirion Evans, Cardiff University

12:00 – 13:25 **Lunch**
Restaurant

- 13:30 – 13:55 **Project B14005: Apply molecular techniques to the nucleic acid archive generated from stool samples archived from the infectious disease study including project B14004: Generate an archive of extracted nucleic acid for IID archived faecal specimens**
Dr Jim Gray, Health Protection Agency and Dr Jim McLauchlin, Health Protection Agency
- 14:10 – 14:25 **Project B18021: The second study of infectious disease (IID) in the community – determining disease burden and calibrating national surveillance data in the United Kingdom**
Professor Sarah O'Brien, University of Manchester
- 14:35 – 14:50 **Mid-afternoon tea and coffee break**
Lounge
- 14:50 – 15:50 **Open discussion including future priorities**
- 15:50 – 16:00 **Conclusions**
- 16:00 **CLOSE OF DAY 1 OPEN SESSION**
- 16:05 – 18:15 **Day 1 closed session for panel members only**
Lecture Room 6
- 19:30 **Dinner**
Restaurant

AGENDA

REVIEW OF THE B14 FOODBORNE DISEASES RESEARCH PROGRAMME

23rd – 25th June 2008

Training and Conference Centre, Radcliffe House,
University of Warwick, Coventry

Day 2: 24th June 2008

Lecture Room 4

09:00 – 09:30 **Registration with morning tea and coffee**
Lounge

09:30 – 09:35 **Chair's Welcome**
Professor Bill Reilly

SESSION III: REVIEW OF THE FSA'S B14 *CAMPYLOBACTER* AND *SALMONELLA* RESEARCH

09:35 – 10:00 **Project B14011: Case-control study of risk factors for *Campylobacter jejuni* infectious intestinal disease (IID) in England and Wales**
Professor Sarah O'Brien, University of Manchester

10:15 – 10:40 **Project B14012: Environmental and waterborne sources of *Campylobacter* in the North West of England and their influence on seasonal human infection**
Professor Eric Bolton, Health Protection Agency

10:55 – 11:10 **Mid-morning tea and coffee break**
Lounge

11:10 – 11:25 **Project B03013: Comparison of the incidence of *Campylobacter jejuni* and *Campylobacter coli* in North West of England and Northern Ireland**
Professor John Threlfall, Health Protection Agency

11:35 – 11:50 **Project B03014: Whole genome analysis fluorescent AFLP – identification of post speciality genes of *Campylobacter* and their value as epidemiological markers**
To be confirmed, Health Protection Agency

12:00 – 12:15 **Project B01013: Genotypic subtyping of multiresistant *Salmonella* Typhimurium DT 104 from food, animals and humans**
Professor John Threlfall, Health Protection Agency

12:25 – 13:45 **Lunch**
Restaurant

SESSION IV: REVIEW OF THE FSA'S B14 ENTEROAGGREGATIVE *ESCHERICHIA COLI* RESEARCH

13:45 – 14:00 **Project B14002: Detection of Enteroaggregative *Escherichia coli* in clinical specimens and foods**
Dr Henrik Chart, Health Protection Agency

14:10 – 14:25 **Project B14003: Development and validation of diagnostic tests for Enteroaggregative *Escherichia coli***
Dr Anna Snelling, University of Bradford

14:35 – 14:50 **Mid-afternoon tea and coffee break**
Lounge

14:50 – 16:00 **Open discussion including future priorities**

16:00 – 16:10 **Conclusions**

16:10 **CLOSE OF DAY 2 OPEN SESSION**

16:15 – 18:30 **Day 2 closed session for panel members only**
Lecture Room 6

19:30 **Dinner**
Restaurant

AGENDA

REVIEW OF THE B14 FOODBORNE DISEASES RESEARCH PROGRAMME

23rd – 25th June 2008

Training and Conference Centre, Radcliffe House,
University of Warwick, Coventry

Day 3: 25th June 2008

Lecture Room 4

09:00 – 09:30 **Registration with morning tea and coffee**
Lounge

09:30 – 09:35 **Chair's Welcome**
Professor Bill Reilly

SESSION V: REVIEW OF THE FSA'S B14 CLOSTRIDIA RESEARCH

09:35 – 09:50 **Project B14006: Assessment of the ability of *Clostridium perfringens* strains to produce toxin after exposure to defined environmental conditions**
Dr Kathie Grant, Health Protection Agency

10:00 – 10:15 **Project B14007: Food safety implications of potentially pathogenic clostridia**
Dr Bernard Mackey, University of Reading

10:25 – 10:40 **Project B14008: Development and validation of a versatile model for predicting the growth of *Clostridium perfringens* during the cooling of meat**
Dr Bernard Mackey, University of Reading

10:50 – 11:05 **Mid-morning tea and coffee break**
Lounge

11:05 – 11:30 **Project B14009: Improved control of *Clostridium perfringens* including Project B13005: Expansion of the Perfringens Predictor model to include pH, nitrite and salt concentrations**
Professor Mike Peck, Institute of Food Research

SESSION VI: FUTURE RESEARCH PRIORITIES

| | |
|---------------|--|
| 11:45 – 13:15 | Open discussion including future priorities |
| 13:15 – 13:25 | Conclusions |
| 13:25 | CLOSE OF DAY 3 OPEN SESSION |
| 13:30 – 14:45 | Lunch Restaurant |
| 14:45 – 16:45 | Day 3 closed session for panel members only Lecture Room 6 |
| 16:45 | END OF RESEARCH PROGRAMME REVIEW |

APPENDIX 2: ATTENDEE LIST FOR THE FSA'S B14 FOODBORNE DISEASES RESEARCH PROGRAMME REVIEW

| DELEGATE | ORGANISATION |
|--------------------------|---|
| Mr Alan Godfree | United Utilities |
| Mr Alan Lyne | ADAS |
| Dr Andrea Patterson | Department for Environment Food and Rural Affairs |
| Professor Andrew Fox | Health Protection Agency |
| Dr Andy Timms | University of Nottingham |
| Dr Anna M Snelling | University of Bradford |
| Dr Anne Maire Grey | Health and Safety Executive |
| Dr Ben Maddison | ADAS |
| Dr Bernard Mackey | University of Reading |
| Dr Bernard Rowe | Tesco |
| Professor Bill Reilly | Food Standards Agency Board Member |
| Mr Bobby Kainth | Food Standards Agency |
| Dr Celia Caulcott | Cooper and Caulcott Ltd |
| Professor Charles Penn | University of Birmingham |
| Professor Christine Dodd | University of Nottingham |
| Dr Christopher Baylis | Campden & Chorleywood Food Research Association |
| Dr Clifton Gay | Food Standards Agency |
| Dr David Tompkins | Health Protection Agency |
| Professor Diane Newell | Veterinary Laboratories Agency |
| Dr Dominic Mellor | University of Glasgow |
| Professor Eric Bolton | Health Protection Agency |
| Ms Gael O'Neill | Food Standards Agency |
| Dr Hannah Jackson | University of Manchester |
| Mrs Helen Long | Food Standards Agency |

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|--------------------------|---|
| Dr Henrik Chart | Health Protection Agency |
| Dr Ian Toth | Scottish Crop Research Institute |
| Dr Jacqui McElhiney | Food Standards Agency Scotland |
| Dr Jane Horne | Food Standards Agency Scotland |
| Mrs Janet Williams | Kent Frozen Foods |
| Dr Jean Banks | NIZO Food Research |
| Mrs Jenny Morris | Chartered Institute of Environmental Health |
| Dr Jim Gray | Health Protection Agency |
| Dr Jim McLauchlin | Health Protection Agency |
| Dr Joanna Topping | Leatherhead Food International |
| Professor John Threlfall | Health Protection Agency |
| Dr Jonathan Fletcher | University of Bradford |
| Mr Joshua Atkinson | Food Standards Agency |
| Mrs Joy Gaze | Campden & Chorleywood Food Research Association |
| Ms Julia Wilson | Hygiene Audit Systems |
| Dr Kathie Grant | Health Protection Agency |
| Ms Kathryn Jackson | University of Manchester |
| Mr Keith Gooderham | Private Veterinary Consultant (Poultry) |
| Dr Kevin Gough | University of Nottingham |
| Ms Kirsten Lawton | Hygiene Audit Systems |
| Miss Lesley Larkin | Department for Environment Food and Rural Affairs |
| Mrs Lisa Marie Winnall | University of Birmingham |
| Dr Lorna Marshall | Food Standards Agency Scotland |
| Ms Madeleine Smith | University of Birmingham |
| Dr Meirion Evans | Cardiff University |
| Miss Melissa Thompson | Hygiene Audit Systems |
| Dr Mike Carter | University of Surrey |

| | |
|--------------------------|---|
| Professor Mike Gasson | Institute of Food Research |
| Professor Mike Peck | Institute of Food Research |
| Dr Mike Stringer | Campden & Chorleywood Food Research Association |
| Dr Nicola Holden | Scottish Crop Research Institute |
| Mr Nicholas Laverty | Food Standards Agency |
| Dr Paul Cook | Food Standards Agency |
| Dr Paul Willets | Food Standards Agency |
| Dr Peter Hawtin | Health Protection Agency |
| Dr Peter McClure | Unilever |
| Professor Peter Silley | MB Consult Ltd |
| Mr Phil Richards | University of Nottingham |
| Dr Phil Robbins | University of Birmingham |
| Dr Roy Betts | Campden & Chorleywood Food Research Association |
| Professor Sarah O'Brien | University of Manchester |
| Mr Shanoor Ali | Food Standards Agency |
| Dr Silvia Alonso Alvarez | Royal Veterinary College |
| Mrs Thelma E Hooper | Alcontrol Laboratories |
| Dr Thomas Quigley | Safefood (Food Safety Promotion Board) |
| Professor Tom Humphrey | University of Bristol |
| Miss Tracy Bird | Food Standards Agency |
| Dr Will Sopwith | Health Protection Agency |
| Professor Will Waites | University of Nottingham |
| Dr Yvonne Boyd | Department for Environment Food and Rural Affairs |

APPENDIX 3: BRIEF DESCRIPTION OF THE FOODBORNE DISEASES CHAIR AND PANEL MEMBERS BACKGROUND AND AREAS OF EXPERTISE

Professor Bill Reilly

Bill Reilly is the former Head of the Gastro-intestinal and Zoonoses Section of Health Protection Scotland and a previous Chairman of the Advisory Committee on the Microbiological Safety of Food (ACMSF). Bill is currently a member of the Board of the Food Standards Agency and Deputy Chair of the Scottish Food Advisory Committee.

Bill research interests have covered the relationship between animals and human health, in particular foodborne infection. Bill also chaired the Scottish Executive Task Force on *E. coli* O157.

Professor Tom Humphrey

Tom Humphrey holds the Chair in Veterinary Bacterial Zoonoses at the University of Bristol, School of Clinical Veterinary Science and heads the Foodborne Zoonoses Group. The main focus of his research is on the interaction between animal welfare and behaviour and susceptibility of pigs and chickens to the major zoonotic pathogens *Salmonella* and *Campylobacter* spp. Tom is one of the co-principal investigators on the Defra/Hefce-funded Veterinary Training and Research Initiative (VTRI) project on social stress in pigs and chickens and subsequent disease susceptibility.

Tom has published over 250 scientific papers and is also a member of the Advisory Committee for the Microbiological Safety of Food (ACMSF) and Chairman of the ACMSF Vulnerable and Surveillance Working Group.

Professor Christine Dodd

Christine Dodd's research is centred in the transmission of pathogens through the food chain and in particular through meat production. This has concentrated primarily on *Salmonella* and *E. coli* (as a monitor strain for *Salmonella*) transmission through red meat slaughterhouses with recent studies focusing on *Salmonella* in pork production and the impact of intervention measures. Christine has particular expertise in the use of sub-species typing of isolates by molecular methods to trace routes of contamination. Other experience is in using non-culture based methods such as 16S PCR DGGE to examine bacterial population structures.

Professor Mike Gasson

Professor Mike Gasson is a molecular microbiologist and Deputy Director (Science) at the Institute of Food Research (IFR) in Norwich. Formerly he was Head of Food Safety Science at IFR and led the strategic development of its current research programme with its emphasis on *Salmonella*, *Campylobacter* and *Clostridium botulinum*. His personal research interests are in: the analysis and exploitation of lactic acid bacteria; the characterisation of the GI tract microbiota in man and animals; the development of novel antimicrobials including lantibiotics and bacteriophage endolysins (enzybiotics).

Professor Charles Penn

Professor Charles Penn graduated in Biochemistry at the University of Liverpool followed by PhD studies on bacterial vaccines at the Wellcome Research Laboratories, Beckenham. He then moved to Professor Harry Smith's group at the University of Birmingham to study bacterial virulence mechanisms. He has worked on gene

regulation and functional genomics (proteomics, transcriptomics) of surface antigens, especially flagella, latterly focussing on the foodborne pathogens *Campylobacter jejuni* and *Escherichia coli*. He also has a strong interest in gastrointestinal tract microbiology.

Charles is currently Editor in Chief of the Journal of Medical Microbiology, a Trustee and governor of the Institute of Food Research, and a member of the BBSRC Agri-Food grants committee.

Dr John Cowden

In 1995, after 10 years with the PHLS's Communicable Disease Surveillance Centre, where he was the first head of the Gastrointestinal Diseases Section, John Cowden moved to the Scottish Centre for Infection and Environmental Health (now Health Protection Scotland) where he is the consultant epidemiologist responsible for HPS's Gastrointestinal Diseases and Zoonoses Programme including surveillance, operational support, research and education. John is a member of numerous national and international bodies concerned with foodborne and other infectious intestinal disease (including the Scottish Food Advisory Committee) and has published widely in the peer reviewed literature.

Dr Dominic Mellor

Dominic Mellor graduated from the Faculty of Veterinary Medicine, University of Glasgow in 1991. After 15 months in mixed veterinary practice, he returned to undertake his PhD studies, completed in 1997. He was Lecturer in Farm Animal Medicine and Production for four years before completing a five-year Wellcome Trust funded study on the epidemiology of VTEC infection in Scottish cattle and people. Dominic was appointed Senior Lecturer in Comparative Epidemiology and Public Health in 2004. Since 2006, he has been independent veterinary consultant to Health Protection Scotland on zoonoses. His interests are in the epidemiology of animal and human diseases.

APPENDIX 4: APPLICATION FOR RESEARCH PROGRAMME RCU-B1 (ROAME A) FOR THE B14 FOODBORNE DISEASES RESEARCH PROGRAMME

RCU-B1

Application for Research Programme Funding

SECTION 1 - GENERAL

| | | | | |
|----|---------------------------------------|--|-----------------|------------------------------------|
| 1. | Proposer's full name and title | DR PAUL COOK | Tel. No. | 020 7276 8950 |
| | Position held | HEAD OF OUTBREAKS & EMERGENCIES, INFECTIOUS INTESTINAL DISEASES, FOOD SAFETY ADVICE BRANCH | E-mail | paul.cook@foodstandards.gsi.gov.uk |
| 2. | Policy division | MICROBIOLOGICAL SAFETY DIVISION | | |
| 3. | Programme title | FOODBORNE DISEASES (B14) | | |

4. **Aim of the research:**

The objective of the Foodborne Disease research theme is to provide robust information on the presence, growth, survival and elimination of micro-organisms throughout the food chain; and the extent, distribution, causes and costs of foodborne disease. Within this theme, research will be commissioned in support of the Agency's strategy to achieve a reduction in the incidence of food-borne disease by 20% over a five-year period.

5. **Abstract of research.**

The overall contribution of food to the burden of infectious intestinal disease (IID) is not known. Outputs from this programme will improve our understanding of the behaviour, physiology and epidemiology of food poisoning organisms and will assist the Agency's foodborne disease strategy and in meeting the target to reduce food-borne disease by 20% over a five-year period.

Campylobacter is the major cause of IID and this organism will be a particular focus of the programme. Work undertaken will include the contribution made by the food chain to the problem of *Campylobacter*. The contribution of foreign travel to the overall burden of food poisoning will also be investigated and an additional focus will be to compare the incidence of disease in different parts of the UK.

C. perfringens is frequently associated with gastroenteritis in humans and the Agency's 5-year strategy identifies *C. perfringens* as one of the five organisms for which action is required to reduce the number of cases. This programme contains research to further our understanding of the physiology and behaviour of *C. perfringens* including toxin production. The programme also contains work on other species of Clostridia that may contaminate food. There is a lack of information on the basic physiology of species such as *C. tertium*, *C. bifementans* and *C. butyricum* and this programme should generate information on their minimum temperature, pH and water activity for growth and their potential to form toxins.

The study of Infectious Intestinal Disease in England (the IID Study) demonstrated that Enterohaggative *E. coli* (EAggEC), which are a diverse group of the enterovirulent *E. coli*, are a significant cause of IID in humans. Detection of EAggEC in clinical or food samples is currently limited to specialist laboratories because of the lack of widely available methods for detecting these bacteria. This lack of routine methodology also means our knowledge of the pathways by which EAggEC are transmitted to humans is limited. The work undertaken in this programme will expand our knowledge of this group of *E. coli* and develop a routine detection method that can be used to screen clinical, food and environmental samples.

The IID Study also resulted in an archive of faecal specimens from both the cases and controls that were involved in the study. To facilitate further work on the samples this programme will establish an archive of the microbial nucleic acid from the archived faecal specimens. This nucleic acid archive will then be investigated using molecular approaches to further identify the causes and burden of illness from IID, particularly in cases where a target organism or toxin was not found during the original study.

6a. Total budget requested (ex VAT)

£

6b. Programme duration in years

6c. Proposed start date

6d. Proposed end date

SECTION TWO – RATIONALE

(see Guidance Notes, Section 2)

7. Summarise the issue(s) to be addressed and the driver(s) behind your request for funding for this research / surveillance.

The Agency has a target to reduce the incidence of food-borne disease by 20% over a five-year period. The foodborne disease strategy has identified 5 organisms against which the target to reduce laboratory reports will be measured: *Campylobacter*, *Salmonella*, *C. perfringens*, *E. coli* O157 and *L. monocytogenes*. In order to reduce food poisoning, research needs to be funded to improve our understanding of the physiology, virulence and epidemiology of these main food poisoning organisms and to develop the methods to do this (e.g. typing methods). This programme is designed to address this in broad terms, for example by investigating options for improving surveillance of gastroenteritis in humans, as well as targeting specific causes of food poisoning, such as outbreaks of *C. perfringens* due to handling errors in food preparation. The programme aims to provide a better understanding of the main food poisoning organisms and a microbiological framework for the development of intervention strategies to reduce foodborne disease.

SECTION THREE – AIM(S) OF RESEARCH TO BE UNDERTAKEN

(see Guidance Notes, Section 3)

8. What objectives will you set in order to guide the research towards reaching your aim. To what extent does the success of one objective depend on the successful completion of another?

How essential is each objective in achieving the overall programme aim(s)?

1. Assess the contribution made by the food chain to the problem of sporadic cases of *Campylobacter* infection in humans, relative to other pathways
2. Determine the contribution made by foreign travel to the overall burden of food poisoning
3. Compare the incidence of *Campylobacter* in different parts of the UK
4. Determine the physiology of lesser known microorganisms that may contaminate food
5. Determine best practice for cooling large bulk meats to reduce outbreaks due to *C. perfringens*
6. Investigate options for improving surveillance of gastroenteritis in humans
7. Evaluate and standardise epidemiological tools for the study of foodborne disease

APPENDIX 5: PROJECTS FUNDED UNDER THE B14 FOODBORNE DISEASES RESEARCH PROGRAMME

A list of Food Standards Agency funded projects to be presented and reviewed at the B14 review meeting is given in the table below.

B14 FOODBORNE DISEASES RESEARCH PROGRAMME

| Research Requirement | Project Code | Project Title | Contractor | Start Date | End Date | Total Cost |
|----------------------------|---------------------------|--|--------------------------|-------------|-------------|---|
| MAFF RRD 1999-2000/ FS31/B | B01013 (FS3114) | Genotypic subtyping of multiresistant <i>Salmonella</i> Typhimurium DT 104 from food, animals and humans | Health Protection Agency | 16-Jun-1999 | 31-Mar-2002 | £151,394.00 |
| Single tender action | B03013 | Comparison of the incidence of <i>Campylobacter jejuni</i> and <i>Campylobacter coli</i> in North West of England and Northern Ireland | Health Protection Agency | 03-Dec-2001 | 31-Jan-2004 | £32,296.30 (contract cost) £26,868.00 (final FSA cost) |
| DH RRD | B03014 | Whole genome analysis fluorescent AFLP – identification of post speciality genes of <i>Campylobacter</i> and their values as epidemiological markers | Health Protection Agency | 01-Jun-2000 | 31-Aug-2001 | £69,080.55 |
| Concept note | B08006 | Ascertainment and enhancement of gastrointestinal infection surveillance and statistics (AEGISS) | Health Protection Agency | 01-Jan-2000 | 31-Mar-2004 | £554,194.00 |
| RRD2/B01/B | B14001 | Food poisoning and foreign travel – a study of population health burden and risk factors for antibiotic resistance | Cardiff University | 01-Apr-2002 | 31-Mar-2005 | £413,031.00 |

| | | | | | | |
|------------|---------------------------|--|----------------------------|-------------|-------------|-------------|
| RRD4/B14/C | B14002 | Detection of Enteroaggregative <i>Escherichia coli</i> in clinical specimens and foods | Health Protection Agency | 01-Oct-2002 | 31-Oct-2005 | £238,588.99 |
| RRD4/B14/C | B14003 | Development and validation of diagnostic tests for Enteroaggregative <i>Escherichia coli</i> | University of Bradford | 01-Jan-2002 | 30-Jun-2005 | £236,284.00 |
| RRD4/B14/A | B14004 | Generate an archive of extracted nucleic acid for IID archived faecal specimens | Health Protection Agency | 01-Jan-2003 | 31-Mar-2008 | £287,668.00 |
| RRD4/B14/B | B14005 | Apply molecular techniques of the nucleic acid archive generated from stool samples archived from the infectious disease study | Health Protection Agency | 01-Jan-2004 | 31-Mar-2006 | £256,260.00 |
| RRD6/B14/B | B14006 | Assessment of the ability of <i>Clostridium perfringens</i> strains to produce toxin after exposure to defined environmental conditions. | Health Protection Agency | 01-Sep-2002 | 30-Nov-2005 | £57,677.00 |
| RRD6/B14/D | B14007 | Food safety implications of potentially pathogenic clostridia | University of Reading | 01-Dec-2002 | 28-Feb-2006 | £159,132.00 |
| RRD6/B14/C | B14008 | Development and validation of a versatile model for predicting the growth of <i>Clostridium perfringens</i> during the cooling of meat | University of Reading | 01-Sep-2002 | 30-Nov-2003 | £51,955.00 |
| RRD6/B14/C | B14009⁸ | Improved control of <i>Clostridium perfringens</i> | Institute of Food Research | 01-Sep-2002 | 31-May-2004 | £126,385.00 |

⁸ Project B14009 was evaluated under the B12 Microbiological Risk Assessment Research Programme Review, which was held in March 2007. Therefore project B14009 will not be evaluated by the B14 expert panel but will be presented for delegates' information. Further information on the B12 Microbiological Risk Assessment Programme Review, including the proceeding, are available at: <http://www.food.gov.uk/science/research/researchinfo/foodborneillness/>

| | | | | | | |
|------------|---------------|---|--------------------------|-------------|-------------|-------------|
| RRD6/B14/A | B14011 | Case-control study of risk factors for <i>Campylobacter jejuni</i> infectious intestinal disease in England and Wales | University of Manchester | 01-Nov-2003 | 30-Apr-2007 | £346,564.00 |
| RRD6/B14/A | B14012 | Environmental waterborne sources of <i>Campylobacter</i> in the North West of England and their influence on seasonal human infection | Health Protection Agency | 01-Mar-2003 | 31-May-2006 | £339,762.00 |

RELATED PROJECTS FROM OTHER RESEARCH PROGRAMMES

| Research Requirement | Project Code | Project Title | Contractor | Start Date | End Date | Total Cost |
|------------------------|---------------------------|---|----------------------------|-------------|-------------|---|
| Single tender action | B13005⁹ | Expansion of the Perfringens Predictor model to include pH, nitrites and salt concentrations | Institute of Food Research | 01-Oct-2005 | 30-Nov-2006 | £91,301.98 |
| RRD14/B18/ B18R0001 | B18021 | The second study of infectious disease in the community – determining disease burden and calibrating national surveillance data in the United Kingdom | University of Manchester | 01-Apr-2006 | 31-Mar-2010 | £4,105,560.58 3,918,670.59 (FSA cost) |

⁹ Project B13005 was evaluated under the B12 Microbiological Risk Assessment Research Programme Review, which was held in March 2007. Therefore project B13005 will not be evaluated by the B14 expert panel but will be presented for delegates' information. Further information on the B12 Microbiological Risk Assessment Programme Review, including the proceeding, are available at: <http://www.food.gov.uk/science/research/researchinfo/foodborneillness/>

APPENDIX 6: RESEARCH REQUIREMENT DOCUMENTS

MINISTRY OF AGRICULTURE, FISHERIES AND FOOD (MAFF) RESEARCH REQUIREMENT DOCUMENT: 1999 - 2000

Published March 1998

ASSESSING MICROBIOLOGICAL HAZARDS AND RISKS (FS31)

Introduction

The objectives of this programme are to increase our understanding of how and where pathogenic microorganisms enter the food chain, and to assess how specific food handling and production processes affect the survival, growth and toxin formation of microorganisms in foods. This will allow Government and industry to assess objectively the hazards and risks from foodborne pathogens and assess the significance in consumer safety in terms of changes in food production processes and the consumption habits of the UK population.

Strategic Research Requirements

It is important to determine the strains of foodborne pathogens that cause disease in man and to compare them with strains found in animals, food and the environment. This information will allow sources of infection in humans to be determined and routes of infection to be traced.

Proposals are invited to address the following:

| |
|--|
| B. A comparison of isolates of the major foodborne pathogens obtained from human infection, animals, food and the environment |
|--|

RESEARCH REQUIREMENT DOCUMENT ISSUE 2: 2001/2002

Published September 2000

ASSESSING MICROBIOLOGICAL HAZARDS AND RISKS (B01, formerly FS31)

Assess the contribution made by foreign travel to food poisoning and other infectious intestinal diseases in the UK including those infections which involve antimicrobial-resistant pathogens

Background

Foreign travel is recognised as a risk factor for food poisoning and other infectious intestinal diseases although the scale of the problem in the UK has not been well defined. Gastrointestinal infections acquired abroad will sometimes include organisms which are resistant to antimicrobials although little appears to be known about the role of these organisms in the problem of antimicrobial resistance in the UK. Information is required on the contribution of foreign travel to food poisoning and other IID to assist the Agency in addressing its target of reducing food poisoning in the UK. At the same time information is also needed on antimicrobial resistance associated with such infections to define the scale of the problem and the contribution it makes to the UK picture.

Research proposals are invited to:

B. Assess the contribution made by foreign travel to the burden of food poisoning and other infectious intestinal disease in the UK and the antimicrobial resistance associated with such infections

Published April 2001

FOODBORNE DISEASE (B14)

Nucleic Acid extraction of archived faecal samples from the Infectious Intestinal Disease Study and further molecular identification of the burden of illness.

Introduction

In response to recommendations by the Microbiological Safety of Food Committee (The Richmond Committee) in 1990, the Department of Health (DH) funded a major study of human infectious intestinal disease in England (the IID Study). This study aimed to ascertain the number of cases of infectious intestinal disease (IID) occurring in the community, the proportion of cases presenting to GPs, and their microbial aetiology. Further information about the study and its findings are available in the published report (Infectious Intestinal Disease Study Executive Committee. Report of the Infectious Intestinal Disease Study in England. 2000. The Stationery Office ISBN 0 11 322308 0), and a report of the seminar on the study. (See our website at <http://www.foodstandards.gov.uk/research/iid.htm>)

One of the results of the study was an archive of faecal specimens from both the cases and controls that were involved in the study. When there was sufficient residual faecal specimen remaining after the full range of investigations had been conducted for the study, a 20% suspension of the faeces was made in a cryoprotective broth and stored at -70°C.

To facilitate further work on the samples we are now seeking to establish an archive of the nucleic acid from the microorganisms in the archived faecal specimens. In the study about 45% of faecal samples from cases with IID in the GP component and 63% of cases in the population cohort, failed to yield a target organism or toxin. We would like to use the nucleic acid archive as a resource to further identify the causes and burden of illness from IID, particularly in cases where a target organism or toxin was not found.

Expressions of interest are invited to:

A. Generate an archive of extracted nucleic acid from the archived faecal specimens. The methodology should extract both DNA and RNA and include provision for maintenance of the resulting archive for as long as the nucleic acid is stable.

Linked to requirement A, we are also inviting separate expressions of interest to:

B. Apply molecular techniques to the nucleic acid archive to further our understanding of the causes and burden of Infectious Intestinal Disease

Method for Detecting Enteroaggregative *E. coli*.

Background

The Enteroaggregative *E. coli* (EAggEC) are a diverse group of the enterovirulent *E. coli* and cause Infectious Intestinal Disease (IID) in humans. EAggEC belong to more than 50 "O" serogroups but are characterised by their possession of a 60 MDa plasmid which encodes for their ability to adhere to Hep-2 cells in a "stacked brick" formation. A Department of Health funded study of IID in England (the IID Study) demonstrated that EAggEC are a significant cause of IID in humans. In the study EAggEC were isolated from 5.1% of cases presenting to GPs and 2.9% of cases in the community. Epidemiological analysis of the IID study data pointed to travel abroad and, for those who had not travelled, eating salad at a restaurant as risk factors for infection by EAggEC.

Detection of EAggEC in clinical or food samples is currently limited to specialist laboratories that conduct cell adhesion assays or use gene probes to detect the aggregative adhesion phenotype. Our knowledge of the pathways by which EAggEC are transmitted to humans is limited by the lack of widely available methods for detecting these bacteria. Work is needed to develop a detection method for EAggEC that can be used to screen clinical, food and environmental samples without the need to refer them to a specialist laboratory for testing.

Research proposals are invited to:

C. Develop a method for detecting Enteroaggregative *E. coli* that is suitable for use in routine clinical and food laboratories.

MICROBIOLOGICAL SAFETY DIVISION RESEARCH REQUIREMENT DOCUMENT 6

Published September 2001

FOODBORNE DISEASE (B14)

Introduction

The objective of the Microbiological Food Safety research theme is to provide robust information on the presence, growth, survival and elimination of micro-organisms throughout the food chain; and the extent, distribution, causes and costs of foodborne disease. Within this theme, research will be commissioned in support of the Agency's strategy to achieve a reduction in the incidence of food-borne disease by 20% over a five-year period. *Campylobacter* is the most important cause of bacterial food poisoning in the UK and reducing these infections will be important if the target is to be achieved. Work is needed to improve our understanding of this organism and the sources and routes of transmission. This will help in focussing control measures in the most appropriate way. Work is also needed to address some gaps in our knowledge relating to *Clostridium perfringens*, one of the five organisms that form the baseline for monitoring the Agency's progress towards the target.

Research requirements

Establish the contribution of the food chain to *campylobacter* infection

Campylobacter is the most frequently isolated bacterium associated with gastroenteritis in humans. It usually causes sporadic cases of infection, although in recent years an increasing number of outbreaks have been described. Epidemiological studies suggest that poultry meat is an important vehicle of infection and surveys, including the recent one by the Agency (see website), have shown that a significant proportion of raw poultry meat for human consumption is contaminated with these bacteria. However, poultry meat only explains a proportion of *Campylobacter* cases and the role of other animal products, other foods, water and non-foodborne exposures is still unclear. A pattern is beginning to emerge from recent studies suggesting that routes of transmission other than food may account for a significant proportion of cases. However, firm evidence that this is the case has not yet been established. The Agency would like to commission research to further our understanding of the epidemiology of *Campylobacter*. Collaborative applications are encouraged to promote well balanced proposals that offer value for money and make use of the best available technology and research approaches.

Research proposals are invited to:

A. Conduct epidemiological studies to assess the contribution made by the food chain, relative to other pathways, to the problem of *Campylobacter* infection in humans.

Clostridium perfringens food poisoning

C. perfringens is frequently associated with gastroenteritis in humans and between 1992 and 1999, 13% of foodborne outbreaks in England and Wales were attributed to this organism, although this is known to be an underestimate of the true burden of illness. The Infectious Intestinal Disease (IID) Study in England¹⁰ confirmed that that *C. perfringens* toxin is an important cause of IID in the community and more commonly in cases presenting to GPs. The highest rate of cases presenting to a GP occurred in the under 2 years age group. The Agency's 5-year strategy for delivering its target to reduce the incidence of foodborne disease by 20% identifies *C. perfringens* as one of the five organisms for which action is required to reduce the number of cases.

The organism is commonly found in low numbers in many foods, especially in meat and poultry. It is known to be associated with foods prepared in bulk where there are inadequate cooling facilities for cooked foods. Slow cooling may allow germination of spores that have survived cooking and rapid multiplication of the organism to an infectious dose. It has been suggested that only strains of *C. perfringens* that have been subjected to repeated heating are able to cause food poisoning and that strains freshly isolated from the environment do not. The Agency would like to commission research to further our understanding of the physiology and behaviour of *C. perfringens*. Collaborative applications are encouraged to promote well balanced proposals that offer value for money and make use of the best available technology and research approaches. For research requirement B, applications should also take account of previously funded research in the area of cooling large bulk meats.

Research proposals are invited to:

B. Investigate whether some strains of *C. perfringens* are more toxigenic than others and determine the role of heat treatment on toxin production.

C. Conduct a study of the behaviour of *C. perfringens* and other microorganisms during cooling of large bulk meats.

Physiology of clostridia

The main species of clostridia that are of concern in the context of food safety are *C. botulinum* and *C. perfringens*. *C. perfringens* is the cause of a much less severe but more common type of food poisoning. The physiology of these clostridia is well documented, but much less is known about other species of clostridia that may contaminate foods, e.g. *C. tertium*, *C. bifermentans* or *C. butyricum*. There is a lack of recent information on the basic physiology of these organisms, e.g. information on their

¹⁰ The Report of the Study of Infectious Intestinal Disease in England, ISBN 0-11-322308-0, has been published by The Stationery Office and is available from The Publications Centre, PO Box 29, Norwich NR3 1GN (Tel: 0870 600 5522). A Summary and Executive Summary of the study can be found on the Food Standards Agency website at the following address:
<http://www.foodstandards.gov.uk/research/iid.htm>.

minimum temperature, pH and water activity for growth or potential to form toxins that might lead to illness.

Research proposals are invited to:

D. Investigate the physiology of selected, lesser-known clostridia that may contaminate foodstuffs.

APPENDIX 7: B01013 (FS3114) PROJECT SUMMARY

Project Code: B01013 (FS3114)

Title: Genotypic subtyping of multiresistant *Salmonella* Typhimurium DT 104 from food animals and humans

Contractor: Health Protection Agency

Cost: £151,394.00

Start Date: 15th June 1999

Finish Date: 31st December 2001

Main Aims of the Project:

In collaboration with the Veterinary Laboratories Agency, to standardise and apply recently-developed molecular methods to sub-divide multiple drug-resistant (MR) strains of *Salmonella enterica* serotype Typhimurium definitive phage type (DT) 104 (= MR DT 104) and related phage types for epidemiological purposes.

The Outcome:

- Analysis by pulsed field gel electrophoresis (PFGE) of 853 isolates of MR DT 104 resulted in the identification of 41 distinct pulsed field profiles within the phage type. As expected 89% of the isolates were profile type Xtm 1. Eighteen of the remaining profiles were represented by a single isolate, while the second most common profile accounted for only 2% of the total. The increase of PFGE types with time, and also of the related phage subtypes 104a, 104b, 104c, U302 and U309 have provided further evidence of the evolutionary divergence of a clonal line of MR DT 104 with time.
- The standardisation of methodologies for PFGE and GyrA Mutation Analysis (GAMA), coupled with the ongoing exchange of molecular subtypes and data between the Health Protection Agency (HPA) and Veterinary Laboratories Agency (VLA) has been very useful for outbreak investigations.
- Plasmid profile typing, again based on a standardised methodology, may still be still useful for outbreak investigations (e.g. for the real-time investigation of the national outbreak of MR DT 104 in the summer of 2000, centred on the West Midlands).
- GyrA mutation analysis (GAMA) proved useful in distinguishing several subtypes, but was only applicable to isolates where low level resistance to ciprofloxacin was present (ca 15 % of MR DT 104 isolates). It should be realised that the method relies on spontaneous chromosomal mutation and therefore does not reflect the clonality seen in other subtyping methods.
- Lipopolysaccharide analysis (LPSA) distinguished only two subtypes within MR DT 104, accounting for 80% and 20% of isolates respectively. This method may be useful in conjunction with other subtyping methods.
- Fluorescent Amplified Fragment Length Polymorphism (FAFLP) analysis had perhaps the greatest potential for subtyping MR DT 104 strains and was able to subtype within different PFGE and plasmid profile subtypes. If the method is to be standardised internationally, further work will be required for the standardisation of DNA extraction procedures and on the enzymes to be used.
- Although the ASSSuSpT penta-resistance gene chromosomally-encoded gene

cassette (now referred to as SGI-1) was identified amongst other *S. Typhimurium* phage types, this was due to evolution within DT 104 rather than horizontal gene transfer.

The implications of this work:

The study has:

- Demonstrated the applicability of molecular subtyping to outbreak investigations of MR *S. Typhimurium* DT 104.
- Emphasised the importance of a multi-method approach – no single methodology covers all eventualities.
- Demonstrated the need for and use of standardised methodologies between the HPA and the VLA for outbreak investigations of *Salmonella*.
- Demonstrated the importance of rapid and accurate transmission of (electronic) data between the two organisations.
- Emphasised the need to continually assess new methodologies and their applicability for outbreak investigations
- Demonstrated that a holistic ‘farm to fork’ approach to molecular epidemiology is very important for the investigation of outbreaks of bacterial food-borne zoonoses.

Publications from this work:

Lawson, A.J., Dessama, M.U., Ward, L.R. & Threlfall, E.J. Multiple resistant *Salmonella enterica* serovar Typhimurium DT 12 and 120: a case of MR DT 104 in disguise? *Emerging Infectious Diseases* 2002; 8: 434-436.

Lawson, A.J., Chart, H., Dessama, M.U. & Threlfall, E.J. Heterogeneity in expression of lipopolysaccharide by strains of *Salmonella enterica* serotype Typhimurium definitive type 104 and related phage types. *Letters in Applied Microbiology* 2002; 34: 428-430.

Horby, P.W., O'Brien, S.J., Adak, G.K., Graham, C., Hawker, J.I., Hunter, P., Lane, C., Lawson, A.J., Mitchell, R.T., Reacher, M.H., Threlfall, E.J. & Ward, L.R. A national outbreak of multi-resistant *Salmonella enterica* serovar Typhimurium definitive phage type (DT) 104 associated with consumption of lettuce. *Epidemiology & Infection* 2003; 130: 169-178.

Lawson, A. J., Desai, M., O'Brien, S.J., Davies, R.H., Ward, L.R. & Threlfall, E.J. Molecular characterization of an outbreak strain of multiresistant *Salmonella enterica* serovar Typhimurium DT104 in the United Kingdom. *Clinical Microbiology and Infection* 2004;10: 143-147.

Lawson, A.J., Stanley, J., Threlfall, E.J. & Desai, M. Fluorescent Amplified Fragment Length Polymorphism subtyping of multiresistant *Salmonella enterica* serovar Typhimurium DT104 (MR DT104). *Journal of Clinical Microbiology* 2004; in press

Walker, R.A., Lawson, A.J., Lindsay, E.A., Ward, L.R., Wright, P.A., Bolton, F.J., Wareing, D.R., Corkish, J.D., Davies, R.H., & Threlfall E.J. Decreased susceptibility to ciprofloxacin in outbreak-associated multiresistant *Salmonella* Typhimurium DT104. *Veterinary Record* 2000; 147: 395-396.

Walker, R.A., Saunders, N., Lawson, A.J., Lindsay, E.A., Dassama, M., Ward, L.R.,

Woodward, M.J., Davies, R.H. & Threlfall, E.J. Use of a LightCycler *gyrA* mutation assay for rapid identification of mutations conferring decreased susceptibility to ciprofloxacin in multiresistant *Salmonella enterica* serotype Typhimurium DT104 isolates. *Journal of Clinical Microbiology* 2001; 39: 1443-1448.

Health Protection Agency (2008)

APPENDIX 8: B03013 PROJECT SUMMARY

Project Code: B03013

Title: Comparison of the incidence of *Campylobacter jejuni* and *Campylobacter coli* in North West of England and Northern Ireland

Contractor: Health Protection Agency

Cost: £32,296.00

Start Date: 1st May 2001

Finish Date: 31st January 2004

Main Aims of the Project:

The harmonisation of methods for the subtyping of *Campylobacter jejuni* and *C. coli* in Northern Ireland and England in order to study differences in the epidemiology of the organism in England, Northern Ireland and Lothian: Evaluation of pulsed field gel electrophoresis as a shared resource

The Outcome:

- The application of serotyping demonstrated that while a large number of serotypes were observed, the majority of isolates from cases of human infection in Northern Ireland, North West England and Lothian belonged to a limited number of different types, HS13 and HS50 being the most common.
- A similar phenomenon was also observed for phage typing, (the dominant types being PT1 and PT2).
- Some significant differences in serotype and phage type distribution were found between isolates between the three regions. For example, the frequency of *C. jejuni* HS50 PT5 isolates identified was higher in Northern Ireland while *C. coli* HS56 PT2 was lower, in comparison to North West England.
- Possible explanations for the differences observed include:
 - Variations in the structure of the food chains in these regions, with food being produced and consumed locally (in a relatively closed food chain) in Northern Ireland in contrast to North West England where food may be more likely to be contaminated with a variety of *Campylobacter* types from imported foods from around the UK and abroad.
 - The size of the catchments populations sampled in each of the areas studied. There were nine hospitals in Northern Ireland referring *Campylobacter* samples for testing, 2 in Lothian and 20 in North West England. This variation in numbers may have produced differences in the reported frequencies of serotypes and phage types represented in some areas.
- The method used for PFGE analysis by the HPA laboratory and Queen's University Belfast (the contractor for Northern Ireland), produced comparable results for some isolates when analysed using Bionumerics software.
- Further standardisation of methodology is vital before the technique is sufficiently robust for inter-laboratory comparisons suitable for epidemiological investigations.

The implications of this work:

Broad based inter-laboratory comparisons using standardised molecular methodology for subtyping may prove useful when linked to detailed epidemiological studies, and may help to elucidate variations in the incidence of campylobacter infections amongst humans.

Publications from this work:

PhD thesis – Craig Swift, 2007 – undergoing revision

Health Protection Agency (2008)

APPENDIX 9: B08006 PROJECT SUMMARY

Project Code: B08006

Title: Ascertainment and enhancement of gastrointestinal infection surveillance and statistics (AEGISS)

Contractors: University of Southampton

Cost: £451,000.00

Start Date: 1st January 2000

Finish Date: 31st March 2004

Main Aims of the Project:

- To develop spatio-temporal statistical modelling of syndromic gastrointestinal disease in a defined geographical area.
- To develop a system for collating and managing disparate data sources from multiple microbiology laboratories in a defined area.
- To develop a system for the display and analysis of epidemiological outputs and other information sources based on a geographical information system platform.
- To develop minimum dataset reporting from Primary Care
- To develop a novel generic hypothesis setting risk factor questionnaire

The Outcome:

- An extensive collaboration was developed between academia, primary care (including NHS Direct) and five microbiology laboratories.
- Novel spatio-temporal statistical analysis was developed and applied using an automated data interface and 24 hour web-based reporting system developed and managed by investigators from University of Lancaster. The work established a risk surface of the study area based on analysis of data derived from routine NHS Direct calls for gastrointestinal symptoms. Statistical models were used to identify areas that had a high probability of statistical evidence of raised frequency of reports within the defined time frame. NHS Direct data were used as information from Primary Care remained inconsistent and sparse.
- Novel data capture and management methods and systems were developed and applied by investigators from University of Southampton. Results from routinely submitted faecal samples to the collaborating laboratories and selected data from calls for gastrointestinal symptoms from NHS Direct were received daily. The daily transmission of data was trapped and cleaned. These data were then transmitted on to Lancaster for analysis by spatio-temporal statistics.
- An interactive website built on geographical information system platform with interrogation facility was developed by the Geodata Institute, Southampton. This enabled the display and analytical output of the statistical model and the microbiology results. Other facilities included the distribution maps of water and sewage. Thus it was possible to assemble overlay displays identifying risks and potential sources with microbiological outputs.
- The display of results of the statistical model and the microbiological output were available within 24-48hours of receipt by the project team.
- Analysis of the statistical model and the microbiology output identified areas of

statistical anomalies where the frequency of calls to NHS Direct exceeded expected with a high degree of probability. These areas were investigated for significant microbiological findings. In several incidents, the microbiology appeared to support the statistical output. However, these outputs are unlinked in that they are derived from independent data streams. Therefore, in the majority of incidents it was not possible to draw any conclusions about the statistical findings and the spatially and temporally associated microbiology results. Associating the statistical and microbiology would possibly require a prospective study where questionnaires are applied to both cases represented within the space time statistical anomaly and the cases within the spatial and temporal associated microbiology. Statistically significant common risk factors found when compared to non related cases from both data streams would suggest a relationship.

- In one incident, the statistical model identified an area in a city centre with significant increase over expected calls to NHS Direct. An examination of the cases that called NHS Direct within the identified area in the defined timeframe showed that they were predominantly aged between 20 and 29 years old. Some days following this event, there were microbiologically confirmed cases of cryptosporidium from the same area as the statistical anomaly. These had the same age distribution as the NHS Direct cases suggesting they may be linked. In a prospective study, once the statistical anomaly had been detected, the GP practices in the statistical anomalous area would be identified and patients presenting to these practices with acute onset of gastrointestinal symptoms without a history of recent travel would be included in the questionnaire application together with a control group from an unrelated practice. It is interesting to note that the NHS Direct cases were identified by the statistical model several days ahead of the microbiology.
- Data were too inconsistent to be a high value from Primary Care, including those captured by electronic means direct from the practice systems. However, this remains an attractive option if the practice systems could be interrogated automatically removing the need for the GP to actively record information and transmit the data for analysis.
- There was a suggestion that different populations used NHS Direct and General practitioners. This was one inference when a cryptosporidium outbreak was confirmed microbiologically, but not detected by the statistical model based on NHS Direct consultations. The majority of microbiologically confirmed cases in this outbreak were very young and one interpretation was that the parents of these patients preferred to consult their GPs rather than NHS Direct. This would need investigation in any further work in this area.

The implications of this work:

This project demonstrated that spatio temporal statistical modelling of non specific gastrointestinal cases could identify anomalous locations for further investigation to detect potential common source outbreaks. The project was successful in developing a system capable of capturing, collating, analysing and displaying surveillance data from different and disparate sources in real-time. It showed the potential for interrogation of data from GP practices, but required further work to remove the need for GPs to contribute manually.

Syndromic surveillance increases sensitivity and may identify associated cases in space and time where traditional microbiological investigation could fail to detect because of

lack of sensitivity and possible erroneous discrimination.

Publications from this work:

On-line Monitoring of Public Health Surveillance Data. Diggle, P., Knorr-Held, L., Rowlingson, B., Su, Ting-Li., Hawtin, P. and Bryant, T.N. In: Monitoring the Health of Populations. Statistical Principles & Methods for Public Health Surveillance. Eds Brookmeyer and Stroup. Oxford University Press 2004.

Health Protection Agency (2008)

APPENDIX 10: B13005 PROJECT SUMMARY

Project Code: B13005

Title: Expansion of the Perfringens Predictor model to include pH, nitrite and salt concentrations

Contractor: Institute of Food Research

Cost: £91,302.00

Start Date: 1st October 2005

Finish Date: 30th November 2006

Main Aims of the Project:

Clostridium perfringens is reported to be the second most common cause of foodborne illness and associated death in England and Wales. Spores of *C. perfringens* are present in low numbers in meat and poultry, and will (in most cases) survive the heat processes used in the manufacture of meat products. If the cooling process is too slow, *C. perfringens* can grow to hazardous levels. Consumption of such products can lead to food poisoning. The key to preventing foodborne illness is to apply a safe cooling procedure.

With previous Food Standards Agency funding (project B14009), we developed and made freely available on the internet, a user-friendly software tool (Perfringens Predictor) that enables the user to determine whether cooling profiles are safe with respect to *C. perfringens*. The user inputs the temperature profile of the meat during cooling into Perfringens Predictor, and the output is a prediction of growth of *C. perfringens* under otherwise optimal conditions (pH 6–7, low sodium chloride concentration, no sodium nitrite).

The present project has been concerned with expanding Perfringens Predictor to enable the user to take account of the pH and sodium chloride concentration of the meat, and whether it is cured. These changes to Perfringens Predictor are designed to contribute to the microbiological safety of food by giving the user a more accurate prediction of growth of *C. perfringens* during the cooling of meats.

The project objectives were:

1. Develop a new dynamic model to predict the growth of *C. perfringens* that takes account of the pH, sodium chloride and sodium nitrite concentration;
2. Validate the new model by demonstrating that it provides a good prediction of *C. perfringens* growth during the heating/cooling of meats;
3. Revise the user-friendly computer software tool to enable the user to input the cooling profile, pH, sodium chloride and sodium nitrite concentration. Also revise the user manual;
4. Make the revised version of Perfringens Predictor available free of charge within ComBase Predictor. Give presentations and publications to ensure that the availability of Perfringens Predictor is widely known.

The Outcome:

- A new dynamic model has been developed for growth of *C. perfringens* that includes temperature (15°C-52°C), pH (5.0–8.0), sodium chloride concentration (0.5–4.0%) and sodium nitrite concentration (0–150 ppm). The model is based on 84 growth curves (extracted from ComBase, the literature, and new growth curves generated at IFR).
- It was demonstrated that the new dynamic model provided a valid prediction of the growth of *C. perfringens* during the cooling of meats, and as affected by pH, sodium chloride and sodium nitrite concentration. This was done by comparing the prediction of growth from Perfringens Predictor (based on the cooling profile, pH, sodium chloride and sodium nitrite concentration of the meat) with observations of growth from twenty new heating/cooling curves carried out with different meats (pork, beef and turkey) at various pH, sodium chloride and sodium nitrite concentrations at IFR, and also with a similar number of observations reported in the literature.
- The user-friendly software tool (Perfringens Predictor) has been revised to enable the user to input pH, sodium chloride concentration or water activity, and sodium nitrite concentration (cured or non-cured options). The user manual has been updated. The software provides the user with a prediction of growth of *C. perfringens* under the specified dynamic cooling conditions. Interpretation advice is automatically brought up after the predictions are displayed. An improved web-based version of the software tool (and user manual) called Perfringens Predictor Web Edition is available at the ComBase website. Several presentations have been made to highlight the availability of Perfringens Predictor, and publications in peer reviewed journals are planned.

The implications of this work:

A new dynamic predictive model has been developed and validated for predicting growth of *C. perfringens* during the cooling of meats that enables the user to take account of the cooling profile, pH, sodium chloride and sodium nitrite concentration. It is suitable for use with cured meat products, and gives a more accurate prediction when pH and/or sodium chloride concentration are not optimal. This tool will be of considerable benefit in determining safe cooling procedures for meat products. Creating a web-based version of this program, that can be accessed via the internet, will allow Perfringens Predictor to be used more easily than the previous Excel-download version, and should contribute to the Food Standards Agency's aim to reduce the number of cases of foodborne illness.

Publications from this work:

Software tool

The main output from this project is the software tool Perfringens Predictor. This software tool is freely available on the internet (ComBase website). The availability of Perfringens Predictor is highlighted on the IFR website. Perfringens Predictor has been and will continue to be demonstrated at ComBase workshops (typically, at least two are held per annum).

Presentations/Publications

Novak, J.S., Peck, M.W., Juneja, V.K. & Johnson, E.A. (2005). *Clostridium botulinum* and *Clostridium perfringens*. In "Foodborne Pathogens: Microbiology and Molecular Biology" Eds P.M. Fratamico, A.K. Bhunia & J.L. Smith. pp. 383-407. Caister Academic

Press, Wyndham, UK.

Baranyi, J., Pin, C., Marc, Y. & Métris A. (2006). ComBase and its Application to Quantitative Microbial Risk Assessment Problems. School of Chemical Sciences and Food Technology, Universiti Kebangsaan Malaysia, February 2006.

Aldus, C.F., LeMarc, Y., Plowman, J., Baranyi, J. & Peck, M.W. (2006). Further improvements in the prediction of growth of *Clostridium perfringens* during the cooling of meat using Perfringens Predictor. Poster presentation at the Med-Vet-Net 2nd Annual general meeting, Malta, May 2006.

Baranyi, J. & Pin, C. (2006). ComBase Workshop. Facultad de Ingeniería de la Universidad de La Sabana, Colombia, October 2006.

Pin, C., Le Marc, Y. & Métris A (2006). Clean Labels, A Fresh Look at Food Safety! LFI Conference, Leatherhead, U.K., November 2006.

(6) Peck M. W., Plowman J., Aldus C. F., LeMarc Y. & Baranyi J. (2007). Development and validation of Perfringens Predictor model. Oral presentation at FSA Microbiological Risk Assessment Programme Review, Reading, U.K., March 2007.

Le Marc Y., Plowman J., Aldus C.F., Munoz-Cuevas M., Baranyi J. & Peck M.W. (2007). Modelling the outgrowth of *Clostridium perfringens* during the cooling of bulked meat. Oral presentation at Proceedings of the 5th International Conference in Predictive Modelling in Food, Athens, Greece, September 2007.

Grant, K.A., Kenyon, S., Nwafor, I., Plowman, J., Ohia, C., Halford-Maw, R., Peck, M.W., and McLauchlin, J. (2008). The identification and characterisation of *Clostridium perfringens* by real-time PCR, location of enterotoxin gene and heat resistance. Foodborne Pathogens and Disease (submitted).

LeMarc, Y., Plowman, J., Aldus, C.F., Munoz-Cuevas, M., Baranyi, J. and Peck, M.W. (2008). Modelling the growth of *Clostridium perfringens* during the cooling of bulked meat. International Journal of Food Microbiology (submitted).

Earlier presentations/publications are listed in the report for project B14009.

Institute of Food Research (2008)

APPENDIX 11: B14001 PROJECT SUMMARY

Project Code: B14001

Title: Food poisoning and foreign travel - a study of population health burden and risk factors for antibiotic resistance

Contractor: Cardiff University

Cost: £413,031.00

Start Date: 1st April 2002

Finish Date: 29th September 2005

Main Aims of the Project:

- To quantify the incidence in the general population of travel-related gastrointestinal illness using population health survey data
- To establish regional primary care sentinel surveillance for travellers' diarrhoea
- To identify risk factors for ciprofloxacin-resistant *Campylobacter* infection using a case-comparison study
- To compare clinical outcome of illness caused by ciprofloxacin resistant and ciprofloxacin sensitive *Campylobacter* infection using a case-comparison study

The Outcome:

Population health survey:

- Nearly 1 in 5 adults reported gastrointestinal illness in the previous 3 months (equivalent to 0.8 episodes per person-year), and 1 in 6 of these consulted a doctor.
- Around 1 in 60 adults had gastrointestinal illness that occurred whilst abroad, equivalent to around 4 million cases of travellers' diarrhoea each year in the UK.
- People with travel-related illness were less likely to consult than those with domestic illness.

Sentinel surveillance:

- Highest rates of travellers' diarrhoea were in summer, in people aged 15-24 years, and in travellers to Southern Europe.
- Risk of travellers' diarrhoea is higher in travellers to destinations outside Europe and North America.

Case-comparison study:

- Travel abroad was the most important risk factor for ciprofloxacin resistant *Campylobacter* infection, especially travel to Spain.
- Travellers with resistant infection were more likely to drink bottled water than travellers with sensitive infection.
- Patients with domestically-acquired, resistant infection were also more likely to drink bottled water than those with domestically-acquired sensitive infection, but no more likely to eat chicken or pre-cooked cold meats.
- Patients with ciprofloxacin-resistant *Campylobacter* infection reported similar duration of diarrhoea and similar rates of medium term (6 months) sequelae.

The implications of this work:

Although postal questionnaire surveys probably overestimate the incidence of gastrointestinal illness, they nevertheless suggest a high burden of domestic and travel-related infection each year. Only a minority of patients consult a doctor, and consulters differ from non-consulters in their personal characteristics. Routine surveillance data therefore not only underestimate illness rates, but also give a distorted impression of the epidemiology of the disease.

Sentinel primary care surveillance has the potential to monitor secular trends in travellers' diarrhoea and to help characterise population groups or travel destinations associated with higher risk.

Foreign travel remains the major risk factor for ciprofloxacin resistant *Campylobacter* infection. The source of infection for domestically acquired, resistant infections appears to be largely the same as for sensitive infections. However, the role of bottled water as a source of both domestically-acquired and travel-related resistant infection needs to be further explored. Ciprofloxacin resistant *Campylobacter* infection does not appear to carry any increased risk of adverse clinical consequences, in contrast to the findings of studies from the US and Denmark.

Publications from this work:

Peer-reviewed publications

Northey G, Evans MR, Sarvotham T, Thomas DRh, Howard AJ. Enhanced surveillance of the aetiology of travellers' diarrhoea presenting to general practice in Wales. *J Infect* 2006; **53**, e94 [P11] (abstract).

Northey G, Evans MR, Thomas DRh, Sarvotham T, Rigby CJ, Hopkins L, Howard AJ. A case-control study of risk factors and clinical outcome for ciprofloxacin-resistant *Campylobacter* infection. *J Infect* 2006;**53**:e94 [P12] (abstract).

Evans MR, Sarvotham T, Thomas DRh, Howard AJ. Domestic and travel-related foodborne gastrointestinal illness in a population health survey. *Epidemiol Infect* 2006; **134**: 686-93.

Northey G, Evans MR, Sarvotham TS, Thomas DR, Howard AJ. Primary care surveillance of travellers' diarrhoea. *BMC Infect Dis* 2007; **7**: 126.

Publications – in preparation

Evans MR, Northey G, Sarvotham TS, Hopkins LJ, Rigby C, Thomas DRh, Howard AJ. Risk factors for ciprofloxacin-resistant *Campylobacter* infection: a case-comparison study. In preparation for *Emerg Infect Dis*

Evans MR, Northey G, Sarvotham TS, Hopkins LJ, Rigby C, Thomas DRh, Howard AJ. Clinical outcome in ciprofloxacin-resistant *Campylobacter* infection: a case-comparison study. In preparation for *Clin Infect Dis*

Presentations and posters

Evans M, Sarvotham T, Thomas D, Howard A. 'Gastrointestinal illness in a population health survey' (poster). Prevention and control of zoonoses: from science to policy,

Liverpool, June 2005

Evans M, Sarvotham T, Northey G, Thomas D, Howard A. 'General practitioner sentinel surveillance of travellers' diarrhoea' (poster). Prevention and control of zoonoses: from science to policy, Liverpool, June 2005

Evans M, Sarvotham T, Hopkins L, Rigby C, Thomas D, Chalmers R, Thomas P, Howard A. 'Aetiology of travellers' diarrhoea presenting to general practice in Wales' (poster). HPA 3rd Annual Scientific Conference, Warwick, September 2005

Northey G, Evans MR, Sarvotham T, Hopkins L, Rigby CJ, Thomas DRh, Howard AJ. 'A case-control study of risk factors and clinical outcome for ciprofloxacin-resistant *Campylobacter* infection' (oral). HPA 3rd Annual Scientific Conference, Warwick, September 2005.

Evans M, Sarvotham T, Thomas D, Howard A. 'Gastrointestinal illness in a population health survey' (poster). 1st International Conf of Journal of Travel Medicine, London, November 2005.

Evans M, Sarvotham T, Northey G, Thomas D, Howard A. 'General practitioner sentinel surveillance of travellers' diarrhoea' (poster). 1st International Conf of Journal of Travel Medicine, London, November 2005.

Evans M, Sarvotham T, Hopkins L, Rigby C, Thomas D, Chalmers R, Thomas P, Howard A. 'Enhanced surveillance of the aetiology of travellers' diarrhoea presenting to general practice in Wales' (poster). Federation of Infection Societies Annual Conference, Cardiff, November 2005

Northey G, Evans M, Sarvotham T, Hopkins L, Rigby C, Thomas D, Howard A. 'A case-control study of risk factors and clinical outcome for ciprofloxacin-resistant *Campylobacter* infection' (poster). Federation of Infection Societies Annual Conference, Cardiff, November 2005

Evans M, Sarvotham T, Northey G, Thomas D, Howard A. 'General practitioner sentinel surveillance of travellers' diarrhoea' (poster). Five Nations Health Protection Conference, Cardiff, May 2006.

Northey G, Evans M, Sarvotham T, Hopkins L, Rigby C, Thomas D, Howard A. 'A case-control study of risk factors and clinical outcome for ciprofloxacin-resistant campylobacter infection' (poster). Five Nations Health Protection Conference, Cardiff, May 2006.

Cardiff University (2008)

APPENDIX 12: B14002 PROJECT SUMMARY

Project Code: B14002

Title: Detection of Enteroaggregative *Escherichia coli* in clinical specimens and foods

Contractor: Health Protection Agency

Cost: £238,588.99

Start Date: 1st October 2002

Finish Date: 31st October 2005

Main Aims of the Project:

The aim of the project was to develop a simple, but sensitive test for the detection of enteroaggregative strains of *Escherichia coli* (EAggEC). A collection of EAggEC, held by the Laboratory of Enteric Pathogens (LEP), was used as a basis for the research and collaborators also helped in isolating new strains of EAggEC by sending samples of patients' faeces to the LEP for screening for EAggEC. An investigation was carried out into the way these bacteria behaved whilst growing in laboratory media, with the aim of examining if EAggEC could be identified by using defined growth conditions. Finally, any promising tests were evaluated by testing samples of foodstuffs and normal human faeces, which had been artificially contaminated with EAggEC. Once identified, a simplified means of detecting EAggEC would enable the rapid diagnosis of patients with a diarrhoeal illness caused by EAggEC. The same test could be used to analyse foods for EAggEC, ensuring the safety of food stuffs particularly those which are consumed raw.

The Outcome:

- Genotypic test for EAggEC were developed based on 3 genes *aat*, *aap* and *aaiA*, and a fourth *uidA* which could be used to confirm the presence of *E. coli*.
- The primers could be applied to the real-time platforms – Taqman and Light-cycler, and were able to detect EAggEC in pure culture, in foods with and without associated competitor organisms and in clinical samples.
- The 140 strains of EAggEC, from the collection held by the LEP, were readily detected by these methods and using patients' faecal samples the test successfully identified strains of EAggEC in 11 of 12 patients, illustrating that the procedure performed well in the presence of normal faecal bacteria.
- The procedures used were also able to detect strains of EAggEC which have been used to artificially contaminate foodstuffs such as lettuce.
- The EAggEC were not found to have unusual growth patterns and a growth medium specific for detecting EAggEC was not identified. With respect to studies involving dispersin, purified dispersin was obtained and used to prepare specific antibodies in a rabbit.
- Tests were developed to successfully identify dispersin being made by EAggEC but a simple method that could be applied in a routine laboratory was not identified.
- Strains of EAggEC were used to 'spike' mineral water, minced beef, cooked ham, milk, lettuce and bean sprouts. These bacteria were found to remain viable and could be detected readily; however, naturally occurring components in minced beef was

found to hinder the detection of EAggEC bacteria.

The implications of this work:

The work has provided the FSA with valuable information relating to the phenotypic attributes and growth characteristic of EAggEC and has established that apart from the Hep-2 adhesion test, there appears to be few, if any, easily-detected phenotypic attributes that delineate strains of EAggEC from other strains of *E. coli*. The work has demonstrated that the PCR primer sequences used in this study could readily detect strains of EAggEC in clinical samples and foods, and could be used in real-time PCR and in micro array formats. The FSA can advocate a highly sensitive procedure for detecting EAggEC which can be set up and performed in any laboratory with basic molecular biology equipment and expertise.

Publications from this work:

Jenkins, C., Chart, H., Willshaw, G.A., Cheasty, T. and Smith, H.R. (2006). Genotyping of enteroaggregative *Escherichia coli* and identification of target genes for the detection of both typical and atypical strains. *Diagnostic Microbiology and Infectious Disease*: **55**, 13 – 9.

Jenkins, C., van Ijperen, C., Dudley, E.G., Chart, H., Willshaw, G.A., Cheasty, T., Smith, H.R. and Nataro, J.P. Use of a micro array to assess the distribution of plasmid and chromosomal virulence genes in strains of enteroaggregative *Escherichia coli*. *FEMS Microbiology Letters*: **253**, 119 – 124.

Jenkins, C., Tempo, M., Chart, H., Cheasty, T., Willshaw, G.A., Phillips, A.D., Tompkins, D. and Smith, H.R. (2006). Detection of enteroaggregative *Escherichia coli* in faecal samples from patients in the community with diarrhoea. *Journal of Medical Microbiology*: **55**, 1493 – 1497.

Health Protection Agency (2007)

APPENDIX 13: B14003 PROJECT SUMMARY

Project Code: B14003

Title: Development and validation of diagnostic tests for Enteroaggregative *Escherichia coli*

Contractor: University of Bradford

Cost: £263,223.00

Start Date: 1st April 2002

Finish Date: 30th June 2005

Main Aims of the Project:

Development of molecular and non-molecular tests for identification of Enteroaggregative *Escherichia coli* (EAggEC) from food and clinical specimens.

- To identify genetic loci that are conserved among pathogenic EAggEC and use these to develop candidate DNA probes and polyclonal antibodies that will serve as the basis for molecular and non-molecular tests.
- To validate candidate test reagents and protocols for the identification of EAggEC in food and diarrhoeal stools.
- In the event that no single genetic marker could be found that is unique to EAggEC, genetic and phenotypic tests would be sought that might have utility for identifying a pathogenic sub-set of EAggEC, or value as a pre-screen or selective factor for EAggEC.
- To estimate the local prevalence of EAggEC among diarrhoeal cases in Northern England, and build a collection of isolates not biased towards positivity with the existing CVD432 EAggEC-associated probe (which lacks sensitivity). Such a collection can be used to study EAggEC properties and evaluate diagnostic tests.

The Outcome:

- Using a collection of EAggEC strains that had been well-characterised and phylogenetically sub-grouped, two strategies were used to identify conserved EAggEC-specific sequence. The first was repetitive element-based PCR-genomic profiling and the second involved screening hot-spots for genomic island insertion. The results of both screens, as well as gene probing with previously described EAggEC-associated genes, demonstrated that the EAggEC category, as presently defined, is genotypically (and hence phenotypically) heterogeneous. Further evaluation of biochemical profiles, enzyme activities, clumping behaviour and relative surface hydrophobicity confirmed this observation. It was thus established that no one marker can be developed as a tool for identifying *all* EAggEC.
- Two novel targets were identified that were conserved within reference isolates of the multilocus-enzyme electrophoresis-defined EAggEC2 phylogenetic lineage. Through genomic profiling, we determined that EAggEC2 strains carry an open reading frame (orf) identical to one carried by enterohaemorrhagic *E. coli* (EHEC) O157. This orf is also present in the majority of diffusely adherent *E. coli* isolates and can be detected using PCR and DNA hybridization protocols developed and evaluated in this study. The orf encoding the protein proved to be very difficult to clone in *E. coli*, suggesting that the protein itself may be toxic to the host cells when expressed in greater than

normal amounts.

- Through the screening for genomic islands, we determined that O-island#28 from EHEC O157 is conserved among EAggEC2 strains. PCR and DNA hybridization tests for a putative outer membrane protein present at the 5' end of this island were developed and evaluated. Work showed that although the mosaic island varies in content between strains, the selected gene serves as a sensitive marker for the presence of the island and the EAggEC2 phylogenetic group.
- Although it showed evidence of toxicity to *E. coli* cells, the outer membrane protein could be detected in outer membranes preparations from *E. coli* strains carrying the gene under control of its own promoter in multicopy. By expressing the protein under the control of the lacZ-promoter and adding a hexa-histidine tag, we were able to purify the protein. It has been shown to be immunogenic in experimental animals, but antibody preparations have not yet been able to detect other EAggEC which possess the protein.
- *E. coli* isolates were collected from 130 travellers returning to the UK with diarrhoea. Adherent *E. coli*, including EAggEC, were found to be highly prevalent. Strains with a classic aggregative adherence pattern were cultured from 24 (18.5%) of the patients. There were reproducible variations in the aggregative adherence patterns among strains and three major aggregative sub-categories were identified. These were the classic aggregative pattern and variant patterns comprised of aggregative-diffuse and a distinctive pattern we have termed 'Pattern X' formation. The distribution of virulence factors and candidate diagnostic targets varied between classic and variant EAggEC. Although no highly sensitive diagnostic targets were identified, the probes with the greatest sensitivity and specificity for classic EAggEC were **pic**, which encodes an autotransporter mucinase, and CVD432, the existing probe. Neither probe could identify up to 10% of variant EAggEC. For these latter strains, one target identified in this study and the *chuA* gene served as better indicators, with limited specificity. During the study, some strains were identified that genotypically had characteristics of both EAggEC and Enterotoxigenic *E. coli* (ETEC), or EAggEC and Enteropathogenic *E. coli* (EPEC).

The implications of this work:

The data from our work has shown that the EAggEC category of *E. coli* includes a diverse mix of strains with no features in common, other than the ability to show aggregation in the HEp-2 assay. Even for this feature, there are marked variations in the pattern seen. It is not possible to have a DNA-based test that would accurately identify all EAggEC, but the project has made progress with developing tests that pick out subgroups of EAggEC, including those currently believed to cause more severe illness. These tests will assist with future studies aimed at delineating and discriminating sub-categories of greatest clinical interest.

The finding of EAggEC in stool samples of 18.5% of patients with traveller's diarrhoea, most of which were negative for previously described diagnostic targets, adds to the concept that EAggEC are important and widespread pathogens, and hence further research into their properties is urgently needed. We additionally determined that surrogate tests that have been proposed such as the 'clump test' are too non-specific, while a probe designed to identify diffusely-adherent *E. coli* recognizes a specific subset of EAggEC strains. Until clinical studies are able to delineate hypervirulent EAggEC-subgroups, EAggEC should be identified by the HEp-2 assay, or where not possible,

multiple genetic targets should be sought. The targets identified in this study increase the repertoire of tests that may be used. Additionally, the project identified some targets, such as *pic*, which have a greater chance of picking up significant EAaggEC strains than earlier-favoured targets.

The EAaggEC category of *E. coli* can include strains that overlap with other pathotypes of *E. coli*. This has implications for the potential mis-classification of *E. coli* isolates, and the estimation of their relative contribution to gastrointestinal illness.

Publications from this work:

Snelling AM, Macfarlane LR, Fletcher JN, Okeke IN. A DNA probe for diffusely-adherent *Escherichia coli* cross-reacts with a subset of enteroaggregative *E. coli*. Submitted.

Macfarlane LR, Fletcher JN, Ashton R, Chapman PA, Snelling AM, Okeke IN. Utility of the CVD432 probe for identification of enteroaggregative *Escherichia coli* amongst isolates from traveller's diarrhoea. *Clinical Microbiology and Infection* **10** (Suppl 3) p. 258 (2004).

In preparation

MacFarlane-Smith LR, Snelling AM, Fletcher JN, and Okeke IN. Inadequacy of the clump test for presumptive identification of enteroaggregative *Escherichia coli*. Research paper

MacFarlane-Smith LR, Fletcher JN, Snelling AM, and Okeke IN. Identification of two chromosomal islands in a subset of phylogenetically-related enteroaggregative *Escherichia coli* strains. Research paper

Snelling, AM, MacFarlane-Smith, LR, Fletcher JN, and Okeke IN. Enteroaggregative *Escherichia coli* in UK travellers. Research paper

University of Bradford (2008)

APPENDIX 14: B14004 PROJECT SUMMARY

Project Code: B14004

Title: Generation of an archive of extracted nucleic acid for IID faecal specimens

Contractor: Health Protection Agency

Cost: £325,058.00

Start Date: 1st April 2003

Finish Date: 31st March 2008

Main Aims of the Project:

The aims of this project were to: assess the detection of DNA and cDNA using PCR and RT-PCR based procedures for specific gastrointestinal pathogens in archived faecal samples from the first infectious intestinal disease (IID) study: generate an archived DNA and cDNA from all the archived IID faecal samples: maintain the archive and assess the stability of DNA and cDNA for target detection during course of the study.

The Outcome:

- Generic cDNA and DNA extraction procedures were established together with PCR assays for the detection of: norovirus, rotavirus, sapovirus, *Salmonella enterica*, *Campylobacter* spp., enteroaggregative *Escherichia coli*, *Clostridium perfringens*, *Cryptosporidium* spp. and *Giardia* spp.
- A pilot study was performed with a statistically significant sample of the previously identified 'positive' and $\geq 73\%$ of norovirus, rotavirus, sapovirus, *Salmonella enterica*, *Campylobacter* spp., enteroaggregative *Escherichia coli*, and *Cryptosporidium* spp were re-detected. The presence of only 68% of the *Giardia* spp and 34% of the *Clostridium perfringens* could be re-confirmed.
- A complete archive of cDNA and DNA for all 4,677 samples was generated and maintained at -80°C .
- With the exception of Giardial DNA, no statistically significant deterioration in the detection rate of viral cDNA, bacterial DNA and *cryptosporidium* DNA was detected over the 5 year period of storage.
- Re-detection by PCR of giardial DNA was obtained from 80% of the extracts after 18 months, and reduced to between 10-20% after 50 months storage.
- The reasons for the reduced target detection of the giardial DNA as compared to other targets are not known and were not investigated.

The implications of this work:

The generation of this archive allowed the successful completion of a related FSA project (B14005) and the feasibility of application of molecular tests to the IID2 project.

Publications from this work:

Amar C.F.L, East C, Maclure E, McLauchlin J, Jenkins C, Duncanson P, Wareing DA. Blinded application of bacteriological culture, immunoassays and PCR for the detection of gastrointestinal pathogens from faecal samples from patients with community

acquired diarrhoea. *Euro J Clin Microbiol Infect Dis* 2004;23:529

Amar CF, East CL, Grant KA, Gray J, Iturriza-Gomara M, Maclure EA, McLauchlin J. 2005 Detection of viral, bacterial, and parasitological RNA or DNA of nine intestinal pathogens in faecal samples archived as part of the English infectious intestinal disease study: assessment of the stability of target nucleic acid. *Diagn Mol Pathol.* 14:90-6.

C.F.L. Amar, C.L. East, J. Gray, M. Iturriza-Gomara, E.A. Maclure, J. McLauchlin Detection by PCR of eight groups of enteric pathogens in 4627 faecal samples: re-examination of the English case-control infectious intestinal disease study (1993-1996). 2007. *Euro J Clin Microbiol Infect Dis*: 26, 311-323

Health Protection Agency (2008)

APPENDIX 15: B14005 PROJECT SUMMARY

Project Code: B14005

Title: Apply molecular techniques to the nucleic acid archive generated from stool samples archived from the infectious disease study

Contractor: Health Protection Agency

Cost: £268,945.00

Start Date: 1st April 2004

Finish Date: 31st March 2006

Main Aims of the Project:

The aims of this project was to test the archived DNA and cDNA extracted from the archived IID faecal samples (FSA Project B14004) with PCR and RT-PCR based procedures for known and putative agents of infectious intestinal disease.

The Outcome:

- Assays were established for the detection of norovirus, rotavirus, sapovirus, *Salmonella enterica*, *Campylobacter* spp., enteroaggregative *Escherichia coli*, *Cryptosporidium* spp. and *Giardia* spp.
- The percentage of cases in which at least one pathogen was detected was increased from 53% to 76% through the use of molecular detection methods.
- The true burden of disease associated with rotavirus and norovirus was established and shown to be significantly higher than that caused by bacteria and parasites
- A high proportion of enteric viruses, bacteria and parasites were detected in the cases as well as the asymptomatic controls.
- Norovirus and rotavirus were more commonly found in children, *Campylobacter* and *Salmonella* were detected in symptomatic teenagers and young adults, Enteroaggregative *E. coli* in all ages and parasites in children (*Cryptosporidium* and *Giardia*) and young adults (*Giardia*).
- Seasonality of infection with norovirus was autumn/winter, rotavirus was winter/spring, *Salmonella* and Enteroaggregative *E. coli* was summer, *Cryptosporidium* was spring and autumn and *Giardia* was spring and summer.

The implications of this work:

The burden of disease associated with virus infections was high and although predominantly in children was found in all age groups. Asymptomatic infection is not uncommon and may provide a substantial reservoir for onward transmission. Although molecular methods for detecting enteric pathogens have been shown to be sensitive and specific, methods that determine the quantity of the pathogen may be required in order to determine the clinical significance of a result.

Publications from this work:

Amar CF, East CL, Grant KA, Gray J, Iturriza-Gomara M, Maclure EA, McLauchlin J. 2005 Detection of viral, bacterial, and parasitological RNA or DNA of nine intestinal pathogens in faecal samples archived as part of the English infectious intestinal disease

study: assessment of the stability of target nucleic acid. *Diagn Mol Pathol*. 14:90-6.

C.F.L. Amar, C.L. East, J. Gray, M. Iturriza-Gomara, E.A. Maclure, J. McLauchlin
Detection by PCR of eight groups of enteric pathogens in 4627 faecal samples: re-examination of the English case-control infectious intestinal disease study (1993-1996). 2007. *Euro J Clin Microbiol Infect Dis*: 26, 311-323.

Health Protection Agency (2008)

APPENDIX 16: B14006 PROJECT SUMMARY

Project Code: B14006

Title: Assessment of the ability of *Clostridium perfringens* strains to produce toxin after exposure to defined environmental conditions

Contractor: Health Protection Agency

Cost: £133,105.00

Start Date: 14th November 2003

Finish Date: 30th November 2005

Main Aims of the Project:

The aims of this project were to obtain data on a range of strains of *C. perfringens* in terms of their potential to produce enterotoxin and cause food poisoning.

The Outcome:

Heat sensitive strains of *C. perfringens* strains possessing plasmid encoded *cpe* genes are capable of causing food poisoning outbreaks in the UK.

The implications of this work:

This work has contributed to our understanding of the epidemiology of *Clostridium perfringens* food poisoning.

Publications from this work:

K. A. Grant, S. Kenyon, I. Nwafor, J. Plowman, C. Ohai, R. Halford-Maw, M. W. Peck and J. McLauchlin: The identification and characterisation of *Clostridium perfringens* by real-time PCR, location of enterotoxin gene and heat resistance. Submitted to Foodborne Pathogens and Disease Dec 2007

Health Protection Agency (2008)

APPENDIX 17: B14007 PROJECT SUMMARY

Project Code: B14007

Title: Food safety implications of potentially pathogenic clostridia

Contractor: University of Reading

Cost: £174,152.00

Start Date: 1st December 2002

Finish Date: 30th November 2005¹¹

Main Aims of the Project:

1. To establish the proportion of *Clostridium butyricum* strains isolated from natural sources that carry the botulinum neurotoxin gene
2. To determine whether *Clostridium tertium* and *Clostridium bifermentans* produce toxins.
3. To define conditions of temperature, pH and solute concentration that will prevent growth of *Clostridium butyricum*, *Clostridium tertium* and *Clostridium barati*.

The Outcome:

- Fewer than one thousand food samples were examined for *C. butyricum* by enrichment in a minimal medium with lactate and acetate as a source of carbon and energy. Selective antibiotics are present in the medium to favour growth of *C. butyricum*. The foods examined were mainly fresh vegetables but also included milk, cream, yoghurt and pâté. Ninety three isolates were tested for the presence of the gene encoding type E botulinum toxin by PCR but no toxin positive samples were detected. Since there were no positives we may assume the real incidence of toxin positive strains from food is less than 1%.
- Weak toxicity against Vero cells was detected in supernatants from five out of six *C. bifermentans* strains and three out of five *C. tertium* strains but, in most cases, it could only be demonstrated by incubating Vero cells for 48h or longer rather than 24h and was readily lost on diluting the supernatant.
- If the levels of toxin needed to produce an effect in Vero cells also caused illness in humans when ingested, then the implication is that very high concentrations of clostridial cells would be needed before food became toxic. If this is so, then food would be obviously spoiled and unpalatable before that stage had been reached.
- Growth limits for *C. butyricum*, *C. barati*, *C. tertium* and *C. bifermentans* were determined in broth incubated anaerobically at 30°C for up to 42 days. The minimum pH values permitting growth depended on the acidulant and strain. Organic acids were more effective at inhibiting growth than HCl as expected. In general the toxigenic strains appear to be less tolerant of acid conditions than the non-toxigenic ones.
- The lowest pH values at which growth of toxigenic and non-toxigenic strains of *C. butyricum* was observed in broth acidified with HCl were 4.1 and 4.2 respectively. The lowest pH values for growth of *C. bifermentans* and *C. tertium* in broth with HCl as acidulant were 4.1 and 4.2 respectively. In the presence of organic acids the

¹¹ Project B14007 was extended to the 28th February 2006 at no extra cost to the Agency

minimum pH for all species varied between 4.4 and 5.0.

- The minimum water activities for growth of toxigenic and non-toxigenic strains of *C. butyricum* were 0.95 and 0.96 respectively. The minimum water activity for growth of *C. tertium*, *C. bifermentans* and *C. barati* was 0.95 for all.
- The minimum growth temperatures of the toxigenic strains of *C. butyricum* (ca 10 - 11°C) were somewhat higher than for non-toxigenic ones (~8°C). The minimum growth temperature for the other clostridia was also about 8°C.

The implications of this work:

- Toxigenic strains of *Clostridium butyricum* do not appear to be commonly found in food.
- Growth of *C. butyricum*, *C. bifermentans*, *C. tertium* and *C. barati* was prevented by water activities that will prevent growth of proteolytic strains of *C. botulinum*.
- *Clostridium butyricum*, *C. bifermentans*, *C. tertium* and *C. barati* can grow at pH values below those that prevent growth of proteolytic *C. botulinum*. Where pH is a critical factor in food preservation allowance must be made for the greater pH tolerance of *C. butyricum* compared with proteolytic *C. botulinum*.
- pH levels that prevent butyric spoilage by *C. pasteurianum* should easily prevent growth of toxigenic *C. butyricum* strains in acid foods.
- Spores of butyric clostridia may sometimes be found in non-acid foods such as milk products; hence it is important to ensure that spores are eliminated from dairy-derived foods that will support their growth.
- From evidence obtained with Vero cells, it seems unlikely that growth of *C. bifermentans* and *C. tertium* constitutes a significant risk factor in foodborne illness.

Publications from this work:

Richard Sherburn, Hamid Ghoddusi, and Bernard Mackey. (2007) Growth limiting conditions for potentially pathogenic clostridia. Poster Presented at Society for Applied Microbiology Summer Conference, Edinburgh. July 2007.

Hamid Ghoddusi, Richard Sherburn and Bernard Mackey (2007) Isolation of *Clostridium butyricum* strains from natural sources and screening the isolates for presence of the type E botulinum toxin gene. Poster Presented at Society for Applied Microbiology Summer Conference, Edinburgh. July 2007.

Two further papers in preparation

University of Reading (2008)

APPENDIX 18: B14008 PROJECT SUMMARY

Project Code: B14008

Title: Development and validation of a versatile model for predicting growth of *Clostridium perfringens* during cooling of meat

Contractor: University of Reading

Cost: £51,955.00

Start Date: 1st September 2002

Finish Date: 31st August 2002¹²

Main Aims of the Project:

1. Develop and test an experimental system to validate growth of *C. perfringens* at all parts of a three dimensional food subjected to heating and cooling.
2. Incorporate *C. perfringens* growth parameters into the Fryer/Bellara model to produce a combined model for predicting growth in three-dimensional solids.
3. Preliminary validation of growth of *C. perfringens* in minced beef and solidified media under a range of dynamically changing heating and cooling conditions
4. Validate growth of *C. perfringens* in a three-dimensional solid under different heating/cooling conditions

The Outcome:

- Model food systems were developed consisting of (i) clostridial growth medium solidified with a gelling agent (gelrite) contained in cylindrical plastic 'sausage' casings or (ii) minced beef packed into casings of the same size. The gelling agent remained solid above 100°C and allowed experiments to be conducted in this temperature range if necessary.
- Computer code was written in MATLAB to solve engineering equations for predicting the dynamically changing temperature profiles within the cylinders during heating. The resultant predictive model was tested experimentally with thermocouples placed in the gelrite or minced beef sausages. Temperatures measured within the sausages at the centre or at different radii were very close to those predicted by the model under a range of different heating and cooling conditions.
- Growth of *C. perfringens* in liquid medium (Reinforced clostridial medium containing minced beef) was followed at a range of constant or changing temperatures and the viable counts compared with values predicted by the Baranyi model. The agreement between predicted growth and experimental results was very good provided the adjustment index parameter was set at 0.01 or in some cases 0.001. There were no major differences in the extent of the lag phase between spore and vegetative cell inocula under these conditions.
- The Baranyi *C. perfringens* growth model was incorporated in the MATLAB temperature predictor model and simulation studies were carried out comparing growth at different regions of a large cylindrical food product under different cooling regimes. Preliminary validation studies with the small sausage gave good agreement between model and experiment.

¹² Project B14008 was extended to 7th November 2003 at no extra cost to the Agency

The implications of this work:

- The ability to calculate internal temperatures and consequent microbial growth during cooling as a function of external temperatures and product size permits simulation exercises that will help to identify critical factors in risk assessment.
- The model can be extended to allow temperature profiles and microbial growth to be simulated in other in other shaped bodies using appropriate numerical methods.
- The results of this project together with those of colleagues at IFR Norwich, CCFRA and HPA will provide the Food Standards Agency with a powerful tool for evaluating the risk of *C. perfringens* growth under different heating and cooling conditions, and will be an educational resource for the Food and Catering Industries and Enforcement Agencies.

Publications from this work:

Alison Kelly, Caroline Loder, Phillip Robbins, Peter Fryer and Bernard Mackey (2004) Development and validation of a versatile model for predicting growth of *Clostridium perfringens* during cooling of meat. Poster Presented at Society for Applied Microbiology Summer Conference, Cork. July 2004.

University of Reading (2008)

APPENDIX 19: B14009 PROJECT SUMMARY

Project Code: B14009
Title: Improved control of *Clostridium perfringens*
Contractor: Institute of Food Research
Cost: £126,385.00
Start Date: 1st September 2002
Finish Date: 31st March 2004

Main Aims of the Project:

Clostridium perfringens is the second most common cause of foodborne illness and associated death in England and Wales. The Food Standards Agency seeks to reduce the burden of foodborne illness, and *C. perfringens* has been identified as one of five pathogens for which a 20% reduction in foodborne illness is sought over a five-year period. *C. perfringens* is present at low numbers in many foods especially meat and poultry, and is frequently associated with food poisoning when cooked foods are cooled too slowly. A particular concern is the safe production of large bulked meats.

This project aims to provide information on appropriate safe cooling regimes for meats to enable the Food Standards Agency to achieve its target of reducing the extent of foodborne illness associated with *C. perfringens*. The new approach taken here is to produce a user-friendly computer software tool to predict growth of *C. perfringens* based on the temperature during cooling. The user (e.g. EHO, food processor) will input a cooling profile for the meat, and the output will be a prediction of whether a critical cell concentration is reached in a product with that cooling profile, and also whether this complies with advice from the Food Standards Agency on the safe production of cooked meats.

The project objectives were:

1. Develop a dynamic growth model to predict the growth of *C. perfringens* for use during the heating/cooling of meats;
2. Demonstrate that the dynamic growth model provides a good prediction of the growth of *C. perfringens* during the heating/cooling of meats;
3. Produce a user-friendly computer software tool (Perfringens Predictor) to predict *C. perfringens* growth during the heating/cooling of meats. Prepare an easy to use manual;
4. Make Perfringens Predictor software and user manual freely available on the internet. Give presentations and publications to highlight its availability.

The Outcome:

- A dynamic model has been developed to predict growth of *C. perfringens* during the cooling of meats. The model relates temperature to growth. The model takes account of the delay to growth resulting from sub-lethal damage received during the heat process.
- The dynamic model provided a valid description of growth of *C. perfringens* observed at IFR for ten heating/cooling regimes representative of those of interest to the food

industry for use with bulked meats, three initial concentrations of *C. perfringens*, and two meats (beef and turkey). The observed increase in viable count from thirteen cooling profiles described in the scientific literature also agreed with predictions from the dynamic model. Thus, the new dynamic growth model provides a valid prediction of the growth of *C. perfringens* during the heating/cooling of meats.

- A user-friendly computer software tool (Perfringens Predictor) has been produced that predicts the growth of *C. perfringens* during the heating/cooling of meats. An accompanying user manual has been prepared. Both are freely available on the IFR website. The user inputs temperature profiles into Perfringens Predictor, and the software delivers a prediction of growth of *C. perfringens* based on the specified dynamic cooling conditions, with interpretation advice (from the Food Standard Agency). Presentations and publications have been made to highlight its availability.

The implications of this work:

The freely available user-friendly computer software (Perfringens Predictor) will enable food processors, EHOs and other users to assess the safety of cooling profiles used with meats (e.g. bulked meats) with respect to *C. perfringens*. If remedial action is necessary, it will also identify what action is required. Adoption of Perfringens Predictor should bring about a reduction in the number of cases of food poisoning associated with *C. perfringens*, and contribute to the Food Standards Agency aim to reduce the number of cases of foodborne illness associated with *C. perfringens* by 20% over a five-year period. This will contribute to an overall reduction in the burden of foodborne illness.

Publications from this work:

The main output is the software tool Perfringens Predictor. This tool is freely available on the internet (ComBase website). The availability of Perfringens Predictor is highlighted on the IFR website, and has been demonstrated at various ComBase workshops (typically two (or more) are held each year). There have been several presentations/publications:

Plowman, J., LeMarc, Y., Aldus, C.F., Baranyi, J. & Peck, M.W. 2004. Dynamic model for prediction of *C. perfringens* growth during cooling of meat. Oral presentation and abstract at "3rd meeting of EU Concerted Action project *Clostridium*". Oslo, Norway. June 2004.

Baranyi, J. & LeMarc Y. 2004. Using static data from ComBase to build a dynamic predictor. Oral presentation at ComBase Workshop, FDA Training Centre, Washington DC, USA. August 2004.

Plowman, J., Aldus, C.F., LeMarc, Y., Baranyi, J. & Peck, M.W. 2004. Better control of *Clostridium perfringens*: a model for prediction of growth during cooling of meats. Invited oral presentation at Food Standards Agency's 3rd Annual Open Meeting on Research. London, UK. November 2004.

Plowman, J., Aldus, C.F., LeMarc, Y., Baranyi, J. & Peck, M.W. 2004. Better control of *Clostridium perfringens*: a model for prediction of growth during cooling of meats. Poster and computer demonstration at Food Standards Agency's 3rd Annual Open Meeting on Research. London, UK. November 2004.

Plowman, J., LeMarc, Y., Aldus, C.F., Baranyi, J. & Peck, M.W. 2004. Dynamic model for prediction of *Clostridium perfringens* growth during cooling of meat. In "Food Microbiology and sporulation of the genus *Clostridium*" Eds. C. Duchesnes, J. Mainil, M.W. Peck & P.E. Granum. pp. 96. University of Liege Press, Belgium.

Peck, M.W. & Baranyi, J. 2004. Perfringens Predictor – a new software tool is now freely available. IFR News 2004.

Plowman, J., Aldus, C.F., LeMarc, Y., Baranyi, J. & Peck, M.W. 2005. A new dynamic model to predict growth of *C. perfringens* during the cooling of meats. Poster presentation at SfAM meeting on "Guess the future, a thing of the past". Norwich, UK. January 2005.

Aldus, C.F., LeMarc, Y., Plowman, J., Baranyi, J. & Peck, M.W. 2005. Improved prediction of growth of *Clostridium perfringens* during the cooling of meat using Perfringens Predictor. Poster and computer presentation and abstract at SfAM conference on "Spore-forming bacteria". Brighton, UK. July 2005.

Peck, M.W. 2005. Perfringens Predictor Highlighted. Science and Innovation 1:05.

More recent presentations/publications are listed in the report for project B13005.

Institute of Food Research (2008)

APPENDIX 20: B14011 PROJECT SUMMARY

Project Code: B14011

Title: Case-control study of risk factors for *Campylobacter jejuni* infectious intestinal disease (IID) in England and Wales

Contractor: Health Protection Agency

Cost: £346,564.00

Start Date: 1st November 2003

Finish Date: 30th April 2007

Main Aims of the Project:

1. To identify risk factors for human indigenous sporadic *C. jejuni* infectious intestinal disease (IID) and determine the proportion of cases of *C. jejuni* IID attributable to food and other exposures through the calculation of exposure specific population attributable fractions (PAFs)
2. To investigate whether different risk factors are involved in the annual rise in incidence during late spring and whether the distribution of important risk factors varies by geographical region.
3. To explore potential associations between different *C. jejuni* subtypes (sero/phagetypes or antibiotic resistant types) and routes of transmission, seasonality and geography
4. To evaluate genotyping as a molecular epidemiological tool for the differentiation of *C. jejuni* strains using a subset of human isolates of known clinical, epidemiological and phenotypic characteristics.
5. To identify genetic determinants of *C. jejuni* that may be associated with different routes of transmission and severity of disease.

The Outcome:

All three studies point to chicken-related exposures as a risk factor for a substantial proportion of human illness with *Campylobacter* infection. The stronger evidence comes from the case-control study, in which the proportion of adult cases attributable to chicken-related exposures was 41%. This was consistent with the preliminary results of the comparative phylogenomics study in which 51% of isolates examined were assigned to the livestock clade (clade A). The major role for chicken-related exposures in human *Campylobacter* infection was also evident in the MLST study. Human isolates were commonly clustered in clonal complexes known to be related to chicken and bovid sources. However, many clonal complexes identified from chicken sources but not from bovids were present in human disease, whilst complexes that were common in bovids but rare in chickens were also rare in human disease. All the results support the fact that chicken-related exposures are major drivers for campylobacter infections in humans.

In the case-control study the positive linear trend seen with increasing 'usual' consumption of chicken was noteworthy. Consumption of chicken increased risk in those who only consumed chicken prepared outside the home and consumption and preparation of chicken in the home appeared to be associated with decreased risk.

Additional important findings in the case-control study were that self-reported previous

Campylobacter infection and recent use of proton pump inhibitors were associated with a greater risk of infection, as was the recent introduction of pet dog into household. Regular consumption of salads, pulses, and unpasteurised milk, and ownership of pet fish were associated with decreased risk.

No major differences were seen with models weighted for non-response.

The implications of the work:

Chicken-related exposures continue to pose a considerable risk to public health, accounting for just over 40% of all cases. Reducing *Campylobacter*-contaminated chicken entering the food chain should have a major impact on lowering disease burden in humans. Since eating chicken outside the home was strongly associated with the risk of illness, advice to caterers about safe preparation of chicken needs to be reinforced. Antacid use accounted for around 10% of all cases and advice to regular consumers of antacids is needed to reduce their risk of acquiring not only campylobacteriosis but other causes of gastroenteritis.

Although based on relatively small sub-samples, our analyses using MLST and comparative phylogenomics data demonstrate the potential for the combined use of genome-based typing methods and detailed epidemiological information for the study of *Campylobacter* and other pathogens. Comparative phylogenomics approaches can potentially help to refine case definitions by identifying sub-groupings of *Campylobacter* that are more epidemiologically relevant than was previously possible with less sophisticated typing methods. In this study, there was evidence that clades A and B represent populations of *Campylobacter* that are defined by source, with clade A being related to livestock strains and clade B being a seemingly more diverse group that also comprises environmental isolates. No environmental risk factors were identified in the epidemiological study but these exposures were not explored in the same depth as were the chicken exposures so it is possible that we missed some environmental risk factors. The fact that we were unable to perform seasonal analyses might be particularly relevant.

A major limitation of genome-based approaches is that there is currently not enough information from an abundant and sufficiently diverse set of source isolates on which to base phylogenetic classifications that can be used for robust epidemiological analysis. As more information is obtained from source isolates, the full potential for combined genome-based and epidemiological analyses will become apparent. Given more robust phylogenetic classifications, methods such as MLST will enable far better classification of exposure for human isolates, by assigning a probability that a given human isolate is derived from a particular source based on its phylogenetic clustering. A current difficulty with epidemiological studies of gastrointestinal pathogens is that measurement of exposure is insufficiently precise. As an example, information on recent chicken consumption can at best only serve as an indicator of the likelihood that an individual consumed chicken that was both initially contaminated and inadequately prepared to the extent that an infectious dose of *Campylobacter* was ingested. A further limitation is that current analyses of case-control study data make the assumption that, for any randomly selected case of *Campylobacter* IID, infection is equally likely to have resulted from any of the putative risk factors on which information has been collected. This is clearly not a reasonable assumption, since some exposures are far more common than others, and the distribution of *Campylobacter* among these is unequal. Complementary use of

phylogenetic and epidemiological information can potentially make far more efficient use of data, by assigning probabilities of exposure to a particular risk factor for individual cases based on the bacterial strain and determining how much support there is for that particular risk factor in the epidemiological data.

Publications from this work:

Conference proceedings (Peer reviewed)

Tam CC, Higgins CD, Rodrigues LC, Owen RJ, Richardson JF, Curnow J, Lamden K, Millership S, Neal K, Patel B, Sheridan P, Wren BW, Al-Jaberi S, McCarthy N, O'Brien SJ. Risk Factors for Reported *Campylobacter* enteritis in England: a case-control study 4th International Workshop on *Campylobacter*, *Helicobacter* and Related Organisms Rotterdam, The Netherlands, September 2007. *Zoonoses and Public Health* 2007; 54 (Suppl 1): 2-3.

Sheppard SK, McCarthy ND, Colles FM, Richardson JF, Cody AJ, Brick G, Meldrum RJ, Elson R, O'Brien S, Owen RJ, Maiden MCJ. *Campylobacter* from Retail Poultry: MLST analysis and the origin of human infection. 4th International Workshop on *Campylobacter*, *Helicobacter* and Related Organisms Rotterdam, The Netherlands, September 2007. *Zoonoses and Public Health* 2007; 54 (Suppl 1): 36

McCarthy ND, Shepherd SK, Richardson JF, Colles FM, O'Brien SJ, Owen RJ, Maiden MC. Developing an epidemiologically robust typing approach to identify, describe and trace outbreaks of *Campylobacter jejuni* infection. 4th International Workshop on *Campylobacter*, *Helicobacter* and Related Organisms Rotterdam, The Netherlands, September 2007. *Zoonoses and Public Health* 2007; 54 (Suppl 1): 153-4.

Academic Journal Papers

Champion OL, Gaunt MW, Gundogdu O, Elmi A, Witney AA, Hinds J, Dorrell N, Wren BW. Comparative phylogenomics of the food-borne pathogen *Campylobacter jejuni* reveals genetic markers predictive of infection source. *Proc Natl Acad Sci U S A*. 2005 Nov 1; 102(44):16043-8.

Health Protection Agency (2008)

APPENDIX 21: B14012 PROJECT SUMMARY

Project Code: B14012

Title: Environmental and water-borne sources of *Campylobacter* in the North West of England and their influence on seasonal human infection

Contractor: Health Protection Agency

Cost: £421,549.00

Start Date: 1st April 2003

Finish Date: 31st July 2006

Main Aims of the Project:

A high percentage of human infections with *Campylobacter* remain unexplained. A significant proportion of these infections go unrecognised as clusters that are probably due to a common source of infection. Incidence in the UK shows a marked seasonality and this may be mediated by environmental factors. This study will identify and investigate clusters in the North West of England and assess the contribution that environmental exposures, including water-borne sources of *Campylobacter*, make to the burden of human disease.

Up-to-date molecular methods will be used to type *Campylobacters* from human cases and identify potential clusters in space and time. Environmental quantification of *Campylobacter* will detect potential seasonal events leading to increased human exposure, such as biofilm breakdown. Epidemiological relationships between clusters and types found in water and animal specimens will be investigated in relation to these events. The correlation of environmental exposures with peaks of human infection and the seasonality of *Campylobacter* types will be analysed using a range of statistical methods, including the novel application of case series analysis.

Objective 01: Application of sequence typing to human infection with *Campylobacter* spp
To obtain sequence type data on an unselected but defined population of northwest England to ascertain the frequency and seasonal distribution of *Campylobacter jejuni* and *Campylobacter coli* strains causing human infections over a three year period. The anticipated data set will comprise over 1,800 isolates and will provide a substantial database of disease causing isolates and will span three seasonal peak incidence periods.

Objective 02: Collection and analysis of environmental samples

To obtain seasonal environmental data of contamination levels from recreational surface waters in drinking water catchments and drinking water supplies to ascertain seasonal influences on the *Campylobacter* microbial load.

Objective 03: Temporal correlation of infection with seasonal events

To determine temporal correlation of infection with seasonal events to assess whether environmental events (e.g. heavy rainfall) significantly impact the burden of human disease through contamination of drinking water or direct environmental exposure using case series analysis.

Objective 04: rapid sequence based typing scheme to identify significant case clusters

To ascertain the utility of a rapid sequence based typing scheme to identify significant case clusters in a defined population in real-time to facilitate and support epidemiological investigations.

Objective 05: Project Management and Dissemination of Information. To manage the programme within the allocated budget

To communicate the findings effectively and informatively to the Food Standards Agency. To disseminate the intellectual property to a public health, scientific audience and to the public through the scientific press, media and through presentations at local, regional and national public and scientific meetings.

The Outcome:

- This study has established for the first time a database of *C. jejuni* and *C. coli* types from a truly population based sample using Multi-Locus Sequence Typing (MLST) for strain characterisation. There were 1,594 cases were included in the study from two distinct study populations, one rural and the other mainly urban. Thirty two clonal complexes were identified with the major complexes being ST-21, ST 257, ST- 828, ST-45 and ST 48. The majority of *C. coli* isolates from human infections belonged to a single clonal complex ST-828.
- This is the first longitudinal study of aquatic and environmental milieus using a real-time PCR based assay for the detection of *C. jejuni* and *C. coli* in water samples. Drinking water and recreational water sources (in both study areas) were examined and isolates characterised by MLST. This is the first study to produce a MLST database of spatially and temporally related isolates from human cases and those from associated environments. There were different relative ratios of *C. jejuni* to *C. coli* in the different geographical locations. *C. coli* were more prevalent from the River Wyre in the rural sampling area. *C. jejuni* ST-21 and ST-45 were represented in both study areas with the latter significantly associated with the aquatic environment. *C. coli* sequence types from water were more genetically diverse and rarely associated with the human cases studied. These were different from the *C. coli* ST-828 associated with human infections.
- This study used a novel case-series analysis to indicate a causal relationship between significant environmental events and human infection. Questionnaires were completed by 1,137 cases and in total matched with 804 MLST characterised isolates. These were investigated for the effect of demographic, symptomology, geographical incidence, seasonality, animal and environmental exposures and travel. The following is a summary of major sequence type associations:
 - ST-353 – an increased association with animal contact, travel outside the UK, leisure contact with countryside and residence in more rural areas and a decreased association with eating out at a hotel or restaurant (compared with all typed cases)
 - ST-206 – an increased association with travel outside the UK, and with eating out (amongst those who had not travelled), both at takeaways and hotels/restaurants
 - ST-574 - an increased association with animal contact, residence in more rural areas, eating out (especially at takeaways) and leisure contact with countryside

- ST-45 – a decreased association with travel outside the UK, an association with early summer increase in incidence, especially in the rural local authorities of the study, an increased incidence in the rural area (statistically significant), increased association with leisure contact with countryside and a decreased association with eating out at a hotel/restaurant. Further work has established a significant link between cases with ST-45 and the environment
 - ST-354 – a decreased association with residence in rural areas, eating out (especially at a hotel/restaurant) and leisure contact with countryside
 - *C. coli* (ST-828) – on the whole, similar levels of association with the exposures described were seen for *C. coli* types as for *C. jejuni* types, but *C. coli* was more often reported from rural and fringe electoral wards and more associated with eating out at hotels or restaurants, while being less often associated with leisure contact with countryside.
- Retrospective analysis of the MLST database of the human cases in conjunction with spatial and temporal analysis indicated that case cluster can be identified. In one instance a cluster of cases was recognisable as being temporally and spatially related, isolates with sequence type belonging to clonal complex ST-443 from Old Trafford. Clonal complex St-353 showed a similar clustering but was also present over a wider geographical area. In addition MLST appears to provide the necessary discrimination and type-ability to identify international clusters for example there are several sequence types which appear to be associated with foreign travel. Furthermore, in a least two instances several cases associated with foreign travel have been identified to a common location and in one instance with a temporal overlap indicating that MLST provides a unique opportunity to identify clusters on an international level with the possibility of informing control measures if performed in real time.
 - The findings have been communicated to the Food Standards Agency and to the public through the scientific press, media and through presentations at local, regional and national and international scientific meetings. (See below)

The implications of this work:

- This study has confirmed the utility of MLST for strain characterisation of *C. jejuni* / *C. coli* and that is a valuable tool for population based investigations of isolates from human and environmental sources. MLST should become the FSA's standard typing tool for all studies. In the wider context of public health the study has highlighted the need for more joined up studies using these microbiological and epidemiological approaches.
- This study has indicated that some *Campylobacter* infections may be associated with environmental exposure but that this is not likely to be the major route of infection. The implication is therefore that foodborne transmission is the major route of infection. This and future studies should help the FSA to establish what the level of foodborne transmission is
- This study has indicated that the epidemiology of *C. coli* is different. Environmental isolates in this study were different from those causing human disease and therefore the implication is that *C. coli* infection is almost always via the foodborne route. Although *C. coli* is responsible for about 10% of human case per annum this still reflects about 5000 cases per year. There is a need for further study of this important pathogen to elucidate the high risk food products associated with infection.

- Imported infection contributes significantly to the burden of disease and whilst there is already evidence that antibiotic resistance to the quinolones is associated with foreign travel we have shown that this is also related to certain clonal complexes and sequence types. To establish the burden of disease by UK acquired infection it is important to be able to exclude those associated with foreign travel and MLST can aid in the identification of such exposures.
- Recognition of *Campylobacter* and outbreaks and clusters of cases has been difficult but MLST in conjunction with active human surveillance can inform this aspect of the epidemiology of infection. As with other enteric pathogens investigation of outbreaks provides intelligence about transmission and for intervention and prevention of infections.

Publications from this work:

Publications

Will Sopwith, Andrew Birtles, Margaret Mathews, Andrew Fox, Stephen Gee, Michael Painter, Martyn Regen, Qutub Syed and Eric Bolton. *Campylobacter jejuni* Multilocus types in Humans, North West England, 2003-2004. *Emerging Infectious Diseases*, 2006;**12**, 1500-1507

Will Sopwith^a, Andrew Birtles, Margaret Matthews, Prof Andrew Fox, Dr Steven Gee, Dr Michael Painter, Prof Martyn Regan, Prof Qutub Syed, Prof Eric Bolton. The identification of a potential environmentally adapted strain of *Campylobacter jejuni*. *Emerging Infectious Diseases*. Submitted 2008

MSc thesis

“Antibiotic resistance in foreign travel-associated *Campylobacter jejuni*” Heather Freeman, Manchester Metropolitan University. Submitted: September 2007

“The detection, identification and molecular epidemiology of *Campylobacter spp* in surface rivers”. Margaret Mathews, Manchester Metropolitan University. Submitted: April 2008

Posters/Presentations

13th International workshop on *Campylobacter*, *Helicobacter* and related organisms September 4-8 2005, Queensland Australia:

Bolton FJ, Birtles A, Mathews ML, Fox AJ, Sopwith WF, Gee S, Kempster J, Painter MJ. Sequence typing for surveillance of *Campylobacter* infection

Mathews ML, Birtles A, Sopwith WF, Bolton FJ, Allen G, Williamson K, Kempster J, Painter MJ. A study of the ecology of *Campylobacter spp* in river water as a source of human infections

Health Protection Agency, Annual Scientific Meeting, September 2005, Warwick University:

Fox AJ, Sequence typing for surveillance of *Campylobacter* infection

Health Protection Agency, Annual Scientific Meeting, September 2006, Warwick University:

Mathews ML. A Molecular Epidemiology Study of *Campylobacter coli* in Human Infections and River Water in North West England

Birtles A. A population based study of *Campylobacter* spp. infection in North West England: MLST typing of clinical isolates over a two year period

Euroheis meeting (poster) Ostersund, Sweden 2003:

Sopwith W. The Influence of Environmental Sources of *Campylobacter* on Human Infection in North West England

5 Nations (oral presentation, plenary), Manchester, November 2004:

Sopwith W. Beyond Chicken: sequence typing to identify sources of *Campylobacter* infection in North West England

United Utilities Public Health Liaison Conference (oral presentation, plenary), February 2005:

Sopwith W. Sources beyond chicken- Multi locus sequence typing of *Campylobacter* in North West England

International Zoonoses Conference (poster), Liverpool, June 2005:

Sopwith W. Sequence typing for surveillance of environmental *campylobacter*

University of Liverpool Medical Microbiology Seminar Series (oral, plenary), Liverpool, Nov 2006:

Sopwith W. Farms, Forks or Fells? Sequence typing of *Campylobacters* in NW England

CHRO (oral, parallel), Rotterdam, Netherlands, Sept 2007:

Sopwith W. Environmentally-adapted strains? Multi Locus Sequence Typing of *C. jejuni* and *C. coli* in surface water and humans

International Zoonoses Conference (poster), Glasgow, Nov 2007:

Sopwith W. Multi locus sequence typing of *Campylobacters* in north west England

Health Protection Agency (2008)

APPENDIX 22: B18021 PROJECT SUMMARY

Project Code: B18021

Title: The second study of infectious intestinal disease (IID) in the community – determining disease burden and calibrating national surveillance systems in the United Kingdom

Contractor: University of Manchester

Cost: £3,472,745.00

Start Date: 1st April 2008

Finish Date: 31st March 2010

Main Aims of the Project:

1. Estimate prospectively the number and aetiology of cases of IID in the population, contacting NHSD/NHS24, presenting to GPs and having stool specimens sent routinely for laboratory examination in the UK.
2. Compare these numbers and the aetiologies with those captured by the UK laboratory reporting surveillance systems and with calls to NHSD in England and NHS24 in Scotland.
3. Determine the proportion of cases of IID likely to have been acquired abroad.
4. Compare the surveillance pyramids from the first and second studies of IID for England.
5. Compare the aetiology of IID in the first and second IID studies for England.
6. Estimate the number of cases of IID in the population of each UK nation, based on recall, via a national telephone survey of self-reported diarrhoea, conducted over two time periods: a period of seven days, and a period of four weeks.
7. Compare the burden of self-reported illness through the national telephone survey with the burden of self-reported illness captured through NHSD in England and NHS24 in Scotland.
8. Compare the prospective and self-reporting methods for estimating IID incidence in the UK, over two time periods: seven days and four weeks.

Achievements so far:

- Favourable ethical opinion received from North West Multicentre Research Ethics Committee and University of Manchester Committee on the Ethics of Research on Human Beings.
- Research sponsorship secured (University of Manchester).
- National Health Service Research and Development approval sought from 54 Research Management and Governance Offices in England and, the Scottish Multicentre Research and Development Management Review, the Welsh Office of Research and Development and the Research and Development Office of Northern Ireland.
- Pilot studies completed.

The implications of the pilot work:

The pilot studies showed that the research methodology works. The major implications arising out of the pilot studies included:

- Inefficiency of third telephone call for unanswered calls in the telephone survey
- Next Birthday method of sampling in households in the telephone survey difficult to operate
- Lower than anticipated participation in the prospective cohort study
- Lower than anticipated invitations from GPs for patients to take part in the GP presentation study
- Difficulty with applying census questions on socio-economic classification in the Telephone Survey and Cohort Study. This proved more of a problem in the Telephone Survey where some individuals became very suspicious about detailed questions about their occupation.

To address these findings protocol changes were made and submitted to the North West Research Ethics Committee and a favourable ethical opinion was received so they have been implemented for the main study.

Publications from this work:

Conference presentations:

4th Annual Meeting: International Collaboration on Enteric Disease Burden of Illness Studies, Rotterdam, September 2007.

10th Annual Conference of the UK Federation of Primary Care Research Organisations and 1st Joint conference of the UK Primary Care Research Network, Cambridge, November 2007.

University of Manchester (2008)