ACM/788

ADVISORY COMMITTEE ON THE MICROBIOLOGICAL SAFETY OF FOOD

INFORMATION PAPER

MICROBIOLOGICAL SAFETY OF FOOD FUNDERS GROUP REPORT *SALMONELLA* REPORT

The attached papers provide an overview of publicly funded research relating to *Salmonella* from 1999 to December 2004. This report has been drafted by the MSFFG Co-ordinator, Dr Celia Caulcott and is based on project information provided by the Members of the Microbiological Safety of Food Funders Group (MSFFG). The interpretation of this information has not been subject to approval by individual contractors. ACMSF Members should note that this report has been published on the Food Standards Agency website.

Secretariat
June 2006

UK Publicly Funded Research Relating to *Salmonella*: Update, December 2005

Research covered from 1999 to the end of 2004

Report to the Microbiological Safety of Food Funders Group

December 2005

UK Publicly Funded Research Relating to *Salmonella*: Update, December 2005

Research covered from 1999 to the end of 2004

OVERVIEW

The report *UK Publicly Funded Research Relating to Salmonella* has been updated for the Microbiological Safety of Food Funders Group (MSFFG). The new report covers research funded by the member organisations of the MSFFG in a total of 192 research projects over the period 1999 to the end of 2004. During this time, the predominant areas of research have been the molecular biology of *Salmonella*, reflecting the availability of various *Salmonella* genome sequences and the pathogenicity of the bacterium and its interaction with different hosts, again with a strong molecular component.

Salmonella is the second most commonly laboratory-confirmed cause of food-borne infections in the UK, after *Campylobacter*. The majority of cases are sporadic incidents, but there are outbreaks affecting numbers of people. In particular these tend to be associated with the consumption of raw or inadequately cooked food, or cooked food cross-contaminated from raw food. Food-borne *Salmonella* primarily represents a public health issue not only because of the number of cases but also because of their severity. The associated mortality is low but significant.

Many different serotypes of *Salmonella* have been identified, several of which are associated with food-borne illness in humans. In the UK, the vast majority of *Salmonella* cases are caused by *S.* Enteritidis and *S.*Typhimurium.

The number of *Salmonella* research projects to the microbiological safety of food in the UK supported by the member organisations of the MSFFG has exceeded the number relating to either *Campylobacter* or *Escherichia coli* O157 (VTEC) during the period of this report.

Significant advances achieved through research

During the period of the report, the understanding of the pathogenicity of *Salmonella* has greatly advanced, in particular based on the molecular biology of the bacterium and comparative genomic studies making use of the available genome sequences. Understanding of gene function and the identification of specific genes important in the process of invasion and colonisation has greatly developed, benefiting from the application of genomic technologies such as DNA microarrays and gene knock-out mutant collections. There has also been progress in the understanding of the host genetics: this has been most notable with respect to poultry.

The research effort to address the reduction and elimination of *Salmonella* from the human food-supply chain has been considerable. In particular, it has supported major activity to reduce the occurrence of poultry-associated salmonellosis, which has been very successful in recent years.

Outstanding issues

Although significant progress has been made in understanding many aspects of the molecular biology of the pathogenicity of *Salmonella*, there does appear to be a need to develop a coherent explanation events in the host animal when it is invaded by a wild-type *Salmonella*. There is a similar need to develop an understanding of the value of vaccines, the various vaccination options and other potential interventions for farm animals, and how to move these forward. The overall conclusion is that the research effort into *Salmonella* is strong, addressing most areas, but that some gaps remain.

LAY OVERVIEW

This report provides an overview of recent research in the UK on *Salmonella*, an important cause of food poisoning.

Salmonella is a very common group of bacteria found throughout the environment including in farm animals, especially poultry and pigs, and capable of infecting humans. Food-borne *Salmonella* is the second most commonly reported cause of bacterial food poisoning in the UK, after *Campylobacter*. The illness is characterised by watery and sometimes bloody diarrhoea, abdominal pain, headache, nausea, vomiting, and fever. Occasionally there can be complications such as septicaemia and septic arthritis and on rare occasions death, but these are uncommon, mostly affecting the elderly.

Transmission of *Salmonella* to humans occurs by consumption of contaminated food, generally of animal origin, or from faecal material from an infected person or animal. Food-poisoning caused by *Salmonella* is particularly associated with meat, poultry and eggs. In addition, there have been some recent outbreaks associated with consumption of lettuce. Reasons for the large number of cases of food poisoning caused by *Salmonella* include the natural levels of occurrence of the bacterium in the gut of food-producing animals, the opportunities for cross-contamination during the poultry, meat and egg production processes (including organic and free-range) and the behaviour of caterers and consumers. It should be noted that food handlers who observe good hygiene practices are very rarely responsible for initiating outbreaks.

During recent years there has been a major research effort in the UK, and internationally, relating to food-borne *Salmonella* and food poisoning, which is reflected in this report. This research has led to an increased understanding of the causes and control of *Salmonella* infections in humans, which in turn has been applied to the food industry and consumer practice. The overall effect has been a marked decrease in the number of cases of *Salmonella* food poisoning in humans in the last few years.

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INTRODUCTION

1. BACKGROUND

1.1 The Microbiological Safety of Food Funders Group (MSFFG) has previously published reports giving an overview of the research funded by member organisations of the MSFFG relating to various food-borne pathogens such as Verocytotoxin-producing *Escherichia coli*[1], *Campylobacter*[2], *Salmonella*[3] *Listeria monocytogenes*[4] and *Yersinia enterolitica*[5]. As part of the ongoing process of considering research with all food-borne pathogens supported by MSFFG members, the group has undertaken an update of its previous review of research on *Salmonella* published in 1999. This updated report covers research undertaken since 1999.

1.2 This report gives an overview of research related to food-borne *Salmonella* undertaken in the UK and funded by members of the MSFFG. It summarises active research in the period from 1999 to the end of 2004 and seeks to set this in the context of other research and issues within the UK and overseas. In addition, an assessment is made of those areas where further research might be needed.

1.3 *Salmonella* are ubiquitous Gram-negative bacteria found throughout the world in a very wide range of animals and environments. The bacteria are important human and animal pathogens as well as being carried without symptoms by many animals. *Salmonella* are one of the most common causes of food poisoning in the UK: only *Campylobacter* infections are reported more frequently[6]. The majority of recorded cases of *Salmonella* in the UK are sporadic although outbreaks occur within the general population and at institutions. The illness is generally associated with ingestion of contaminated food and symptoms are characterised by nausea, vomiting, diarrhoea, abdominal cramps, headache and fever. Complications include septicaemia or focal infection such as septic arthritis.

1.4 There is significant under-ascertainment of food-borne gastro-intestinal infections. On this basis, a method has been developed to estimate more accurately the morbidity and mortality of disease due to various food-borne pathogens. *Salmonella* are estimated to cause just over 3% of food-borne infections in England and Wales (Adak *et al* (2002)) (See Appendix 1). Of these, approximately 0.3% of cases were fatal. This study, and that of Mead *et al* (1999) demonstrate that *Salmonella* account for more deaths that any other identified food-borne pathogens. It is estimated that of 1800 food-borne deaths due to known pathogens in the US, *Salmonella* were responsible for 31% (Mead et al (1999)) and amongst the 480 deaths due to indigenous

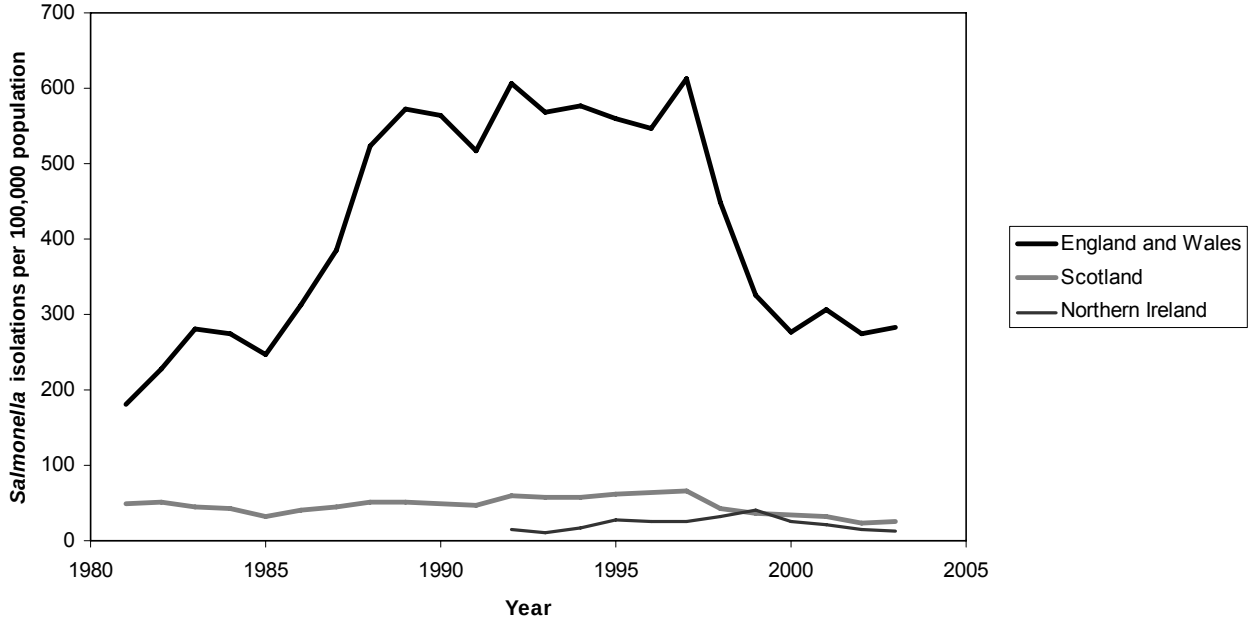
[1] http://www.food.gov.uk/science/research/researchinfo/foodborneillness/microfunders/vtec
[2] http://www.food.gov.uk/science/research/researchinfo/food-borneillness/microfunders/campylobacter
[3] http://www.food.gov.uk/science/research/researchinfo/foodborneillness/microfunders/msffg/55669
[4] http://www.food.gov.uk/science/research/researchinfo/foodborneillness/microfunders/listeria
[5] http://www.food.gov.uk/science/research/researchinfo/foodborneillness/microfunders/yersinia
[6] http://www.hpa.org.uk/infections/topics_az/campy/data_ew.htm

foodborne disease in 2000 in England and Wales, 25% were attributable to *Salmonella* (Appendix 1, Adak et al (2002)).

1.5 There are many different serotypes of *Salmonella* and more than 2460 have been characterised[7]. Of these, *Salmonella* Enteritidis is currently the most common serotype found associated with human salmonellosis in the UK (Figure 2). There was a significant increase in the reported number of *Salmonella* infections in the UK during the 1980s, which was mainly due to an increase in the number of *S.* Enteritidis infections. The other common serotype of *Salmonella* of public health significance is *Salmonella* Typhimurium (until the mid-1980s the most frequently isolated serotype) and specific phage types of both *S.* Enteritidis and *S.* Typhimurium.

1.6 Further significant serotypes of *Salmonella* which infect humans include *Salmonella* Typhi and *Salmonella* Paratyphi, which cause typhoid or typhoid-like fever. Infections with both pathogens are almost exclusively acquired overseas, generally after consumption of heavily contaminated water, and few cases are reported in the UK. Given concern about the increase in antimicrobial drug resistance amongst pathogens, it is also notable that several serotypes of *Salmonella*, including *S.* Typhimurium, *Salmonella* Virchow and *Salmonella* Hadar are often resistant to multiple antibiotics.

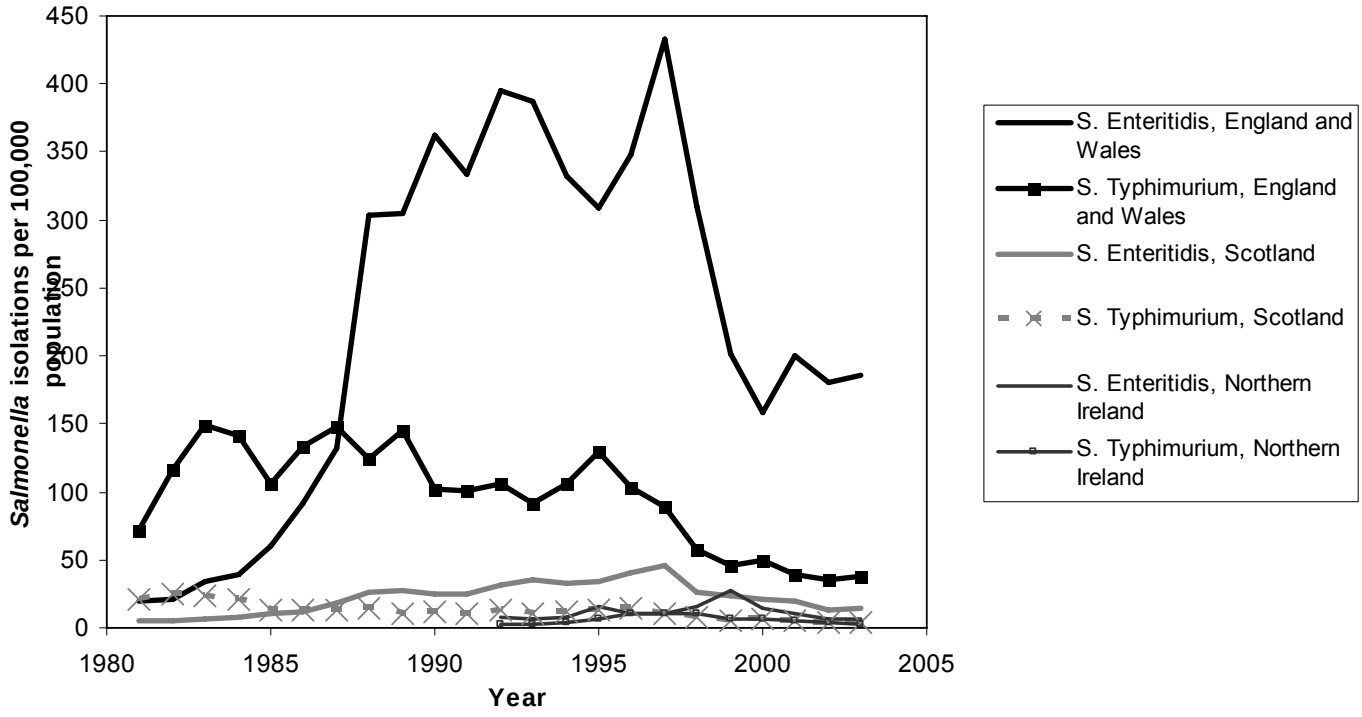
Figure 1: Laboratory reports of faecal isolates of *Salmonella* species, United Kingdom, excluding typhoids and paratyphoids



Data can be found in Appendix 2, Table 3

[7] http://www.hpa.org.uk/infections/topics_az/salmonella/menu.htm

Figure 2: Laboratory reports of faecal isolates of *S.* Enteritidis and *S.* Typhimurium, United Kingdom



Data can be found in Appendix 2 Table 3

1.7 Farm animals, especially poultry and cattle, represent an important reservoir of *Salmonella*. Many animals are carriers of the bacteria, showing no sign of infection. There is considerable opportunity for cross contamination between animals and introduction of the bacteria to the food-supply chain. During the last twenty years concerns about the increased occurrence of food-poisoning caused by *Salmonella* led to significant effort to determine how *Salmonella* are introduced to humans and how the food-supply chain may be managed so as to reduce the likelihood of this. Furthermore, the planned changes in the rules relating to older cattle entering the human food-supply chain[8] may have a significant effect on the pattern of occurrence of *Salmonella* and therefore of the incidence of infections in humans.

1.8 The focus of this report is on research with the principal serotypes of *Salmonella* which are found in the food-supply chain within the UK and which cause food-borne, gastro-intestinal illness in humans. These are primarily *Salmonella* Enteritidis and *Salmonella* Typhimurium. However, there is reference within the report to other strains of *Salmonella*. Many of these can cause human illness, are used within the overall research effort into the

[8] http://www.food.gov.uk/foodindustry/meat/otmreview/
http://www.defra.gov.uk/news/2004/041201a.htm

organism, and data from such projects does contribute to the general understanding of the organism and its relationship with its hosts.

2. NOMENCLATURE

2.1 When first identified, *Salmonella* were named according to the disease they caused or the animal from which they were isolated. This approach changed to naming antigenically distinct types after the geographical location at which a strain was first isolated. With time, and the relatively recent introduction of detailed DNA homology studies, the identification and naming of *Salmonella* has become increasingly unclear, culminating in the simultaneous use of two different nomenclature systems[9] (Tindall *et al* (2005)). Prior to this, the most commonly used system was that of Le Minor and Popoff (1987). A proposal for rationalising this situation has been described (Tindall *et al* (2005)) which clarifies the relationship between the various subspecies and serovars of relevance to intestinal disease in humans and animals.

2.2 The nomenclature in the majority, if not all, of the projects addressed in this report follows that of Le Minor and Popoff (1987), which divides the bacterial species *Salmonella enterica* into six subspecies: *enterica, salamae, arizonae, diarizonae, houtenae* and *indica.* In practice, most of the many serotypes of *Salmonella* which have been identified fall within the species *Salmonella enterica* subsp. *enterica* including serotypes originally referred to as *Salmonella typhimurium*, *Salmonella enteritidis, Salmonella paratyphi,* and *Salmonella typhi.* These are now defined as, for example, *Salmonella enterica* subsp *enterica* serotype Typhi, which may be shortened to *S.* Typhi. This is the common practice and isused throughout this report.

3. METHODS

3.1 This report is based, as was the 1999 MSFFG *Salmonella* Report, on those projects which are funded by the member organisations of the MSFFG. At the time of writing this report, these were the Food Standards Agency (FSA), the Department for Environment, Food and Rural Affairs (Defra), the Biotechnology and Biological Sciences Research Council (BBSRC), the Department of Health (DH), the Department of Agriculture and Rural Development, Northern Ireland (DARD), FSA Scotland, FSA Wales, FSA Northern Ireland, the Food Safety Protection Board (FSPB), the Scottish Executive Environment and Rural Affairs Department (SEERAD) and the Scottish Executive Department of Health (SEDH).

3.2 The MSFFG project database[10] was used to identify projects for inclusion in this report. The database was searched using the term “Salmonella”,

[9] http://www.bacterio.cict.fr/salmonellanom.html#approvedlists

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

identifying all projects which included the term in the title, key words or any component of the text that was available for searching. Projects which were completed by 1st August 1999 were not included. A further search on the BBSRC Oasis database was also carried out, using the key word "Salmonella". Additional relevant projects identified through project reports accessed from the MSFFG database were also checked. Overall, this gave a total of 161 distinct projects for inclusion in this report: a full list of the projects is provided at Appendix 2.

3.3 Studentships have been omitted from consideration.

3.4 Research funded by other agencies, including the Wellcome Trust, NHS Scotland, Health Protection Agency (HPA) and the Medical Research Council (MRC), as well as international research is not included within the body of the report. However, a summary of research funded through these bodies is given in Section 4 below.

4. RESEARCH FUNDED BY OTHER FUNDING BODIES

Within the UK

4.1 The MRC has two projects found in their research database through searching on the term "salmonella". A similar search of the Wellcome Trust grants databases for the period 2000 to 2004 showed seventeen projects: overall these were not related to food-borne *Salmonella*.

4.2 The research projects funded by the Wellcome Trust include the sequencing of the genomes of six *Salmonella* serotypes by the Sanger Institute[11]. These are *S.* Enteriditis PT4, two different strains of *S.* Typhimurium (DT104 and SL1344), *S.* Typhi, *S.* Paratyphi and *Salmonella gallinarum*. This is an important and significant addition to the research effort with all *Salmonella* as the genome sequences underpin many aspects of research with the bacteria.

Within Europe

4.3 The European Union supports research relating to *Salmonella* across similar areas to those addressed within the UK. The Cordis database includes at least 68 research projects relating to *Salmonella* of which at least 35 relate to *Salmonella* in food[12].

Within the USA

4.4 In the United States, federal funding of *Salmonella* research relevant to food-borne illness is supported by the National Institutes for Health (NIH), the US Department of Agriculture and the Food and Drug Administration. The NIH currently has 180 projects recorded in its database which relate to

[11] http://www.sanger.ac.uk/Projects/Microbes/
[12] http://ica.cordis.lu/search/index.cfm

Salmonella[13]. Not all of these are relevant to food-borne illness although a significant number of the projects consider pathogenicity and the host-pathogen interaction. In addition, the US Government supports the Food Safety Research Information Office[14]. Its web site database includes 194 projects related to *Salmonella*, some of which are not funded within the US.

[13] http://crisp.cit.nih.gov/
[14] http://www.nal.usda.gov/fsrio/index.htm

MSFFG FUNDED PATHOGENIC *SALMONELLA* RESEARCH 1999 TO THE END OF 2004

5. DETECTION, DIFFERENTIATION AND DIAGNOSIS

5.1 For any understanding of the relationship between a bacterium and its host, there is a need to be able both to detect the bacterium and to differentiate among types within the species. Methods for the detection and differentiation of *Salmonella* serotypes are well established, with recognised techniques being used within the food industry and the research community. However, there is a constant need to ensure that the best techniques are available, and that best practice is followed. Taking advantage of the various advances in the genomics and genetics of *Salmonella*, there is an important place for ongoing consideration of new approaches to both detection and differentiation, in particular using molecular biology techniques.

5.2 It is also important to ensure that methods are explored for the quantification, separation and recovery of *Salmonella* from foodstuffs. There is a possibility that, in the absence of such techniques, the detection of the presence of *Salmonella* in food will be dependent on enrichment approaches which, whilst effective do not enable the quantification of the occurrence of the bacterium. In addition, there are some environments from which it is not easy to culture *Salmonella* directly, such as nutrient depleted samples. For these, separation techniques are needed that allow the detection of *Salmonella* after separation of the bacteria from other components of the samples.

5.3 Overview of current research

Detection

5.3.1 There is considerable variation in the testing methods and procedures for isolating and detecting *Salmonella* (**FSA ZB00023**) in the poultry industry. For on-farm testing, research has identified boot swabs (of farm workers) and direct litter sampling or swabbing as the most effective methods for detecting *Salmonella* and it suggested that improved swabbing methods should be used in abattoirs (**FSA B15003**). There is similar research to investigate standardisation of sampling and analysis for bacteria in poultry abattoirs (**FSA M01017**). In project **FSPB 00-RESR-046,** bulk tank milk filters were used to detect selected bacteria in raw milk at farm level.

5.3.2 One project has investigated the use of pathogen-specific antibodies to separate different food-borne pathogens from a food homogenate (**FSA FS1319)**. The proposed bacterial trap was initially developed using *Salmonella* and *Listeria*. It was found that the method, using the Dynabead® system to support the antibodies, showed appropriate levels of sensitivity for routine use. A version of this approach has been used in the development of an automated immunomagnetic separation ELISA (Duncanson *et al* (2003))[15].

[15] http://www.ehj-online.com/archive/2000/may2004/may3.html

5.3.3 Several projects have been funded which address the detection of *Salmonella* within the food-supply chain. These have included the use of immunomagnetic nanoparticles to detect *Salmonella* without any need to isolate or culture the bacteria (**SEERAD UMA/001/95**) and techniques dependent on molecular biology, in particular using genomic information (**FSPB 00-RESR-046**, **FSA B09008**, **Defra VM02105**). A polymerase chain reaction (PCR) method has been successfully developed for use with enrichment broths of food-borne bacteria (**B09008**) and both multilocus sequencing typing (MLST) and pulse field gel electrophoresis (PFGE) approaches have been used in identifying the presence of antibiotic-resistant strains (**VM02105**).

5.3.4 Work in project **SEERAD urg/001/96** investigated whether it is possible to distinguish between viable and non-viable cells isolated from food matrices.

5.3.5 The successful culture of isolated bacteria is essential for further characterisation and it is important that there is awareness of the usefulness of different culture media for the growth of *Salmonella*. It was found (**DANI 9642**) that there was considerable variation in the growth of different *Salmonella* serotypes in commercial culture media, and that growth at 35°C was poor in every case.

Differentiation

5.3.6 The differentiation of strains of any pathogenic bacterium is valuable for epidemiological studies. For practical purposes, serotyping, antimicrobial sensitivity testing and phage typing, supported by plasmid analysis and PFGE are often sufficient for analysis of human samples, the Kauffmann-White scheme being the established method for *Salmonella*[16]. However, the advances in molecular biology, including the publication of various bacterial genome sequences suggest that it would be possible to develop rapid, efficient and accessible methods for routinely analysing genomic diversity for *Salmonella*. Work through several research projects has investigated this including **BBSRC D13414/D13422, Defra OZ0311, OZ0312 and FSA B03001, B01009**. A variety of different molecular typing techniques, combined with two electrophoretic techniques were compared (**OZ0311**). Of the methods used, it was concluded that amplified fragment length polymorphism (AFLP) typing was an appropriate genotyping method, able to discriminate between different *Salmonella* serotypes and which could be developed to give sufficient reproducibility. Some reservations were expressed as to the improvements the methods offered over conventional serotyping, and the suggestion was made that microarray analysis could provide a more robust mechanism (**OZ0312**).

[16] http://www.hpa.org.uk/srmd/div_cdmssd_gpbu/salmonella.htm

5.4 Gaps in currently funded research

5.4.1 In the 1999 MSFFG *Salmonella* Report, it was noted that there was a need for work to determine whether molecular methods could be used in order to support epidemiological studies. Some of these methods are now in use, but other molecular typing methods are becoming available and increased understanding of the genetics of the bacteria is providing new insight into the relationships between the different *Salmonella* types. As a single method for identification of all strains of *Salmonella* is unlikely to be found there is a continuing need to explore possible new methods for differentiating the many *Salmonella* strains. This could include evaluating new technologies such as microarray typing.

5.4.2 A further issue identified in the 1999 MSFFG *Salmonella* Report was the need for appropriate improved, standardised and validated methods for sampling at the point of slaughter and for foods. This area is being extensively addressed. However, there is evidence that further work to develop robust, reliable and universal culture methods for *Salmonella* after isolation from food or animal samples would be valuable.

6. MICROBIAL PHYSIOLOGY AND GENETICS

6.1 The study of the genetics and physiology of *Salmonella* is aimed at understanding the function of genes and the interaction of the bacterium with its environment. Much of research of this type is aimed at understanding mechanisms of pathogenesis and virulence, and is covered in the relevant sections of this report. Projects that focus on fundamental aspects of the bacterium, or provide underpinning tools for use by the research community are described in this section.

6.2 All the work on the genetics and physiology of *Salmonella*, and many of the studies of epidemiology and pathogenesis, have benefited from the increasing availability of the genome sequences of many species, serotypes and strains of *Salmonella*[17]. The genome sequences are enabling the creation of sets of gene knock-out mutants and DNA microarrays. These resources are enabling far more rapid progress in understanding of the genetics, physiology and general pathogenesis of the bacterium, to the general advancement of the field.

6.3 Overview of current research

Survival in the environment

6.3.1 Stresses such as heat or acid treatment, as would be experienced by *Salmonella* in the food-supply chain, are likely to have an impact on the genetic properties of *Salmonella* populations. It was noted that high levels of tolerance of acid, heat, air-drying and peroxide correlated with the presence of

[17] http://www.sanger.ac.uk/Projects/Microbes

a major stress response protein (RpoS) whereas tolerance of UV light and growth characteristics at low temperatures did not (**FSA B01007**). It was also found that in natural populations there was a loss of heat resistance in some strains due to a mutation in the RpoS gene. After subjecting the bacteria to heat cycling, both RpoS positive and negative subpopulations showed increased heat resistance. This did not affect the bacterial resistance to high pressure or salt concentration (**FSA B01009**). In a parallel project (**FSA B01010/B01011**) *S.* Enteritidis was exposed to repeated cycles of acid stress which led to an increased resistance to acid; the link between this and RpoS expression was to be investigated. Other genes are also involved in the stress response and the role of several of these in the extracytoplasmic stress response of *Salmonella* was studied (**BBSRC PRS12222**).

6.3.2 Physical stresses such as high pressure or treatment with electric fields may cause significant membrane damage as a primary mechanism of cell death. The interaction between cell membrane damage achieved through these mechanisms and other relevant factors such as pH and osmotic stress was investigated in order to determine the overall impact on cellular viability (**FSA FS1532**).

6.3.3 A very specific and potentially problematic response to stress is the formation of viable, non-culturable cells. Some work has been undertaken to investigate the infectivity of such cells formed in response to nutritional stress (**BBSRC D06412**).

6.3.4 The survival of several *Salmonella* serotypes in dry conditions found that *S.* Enteritidis phage types PT4, PT6 and *S.* Typhimurium DT104 were no better able to survive than other *Salmonella* serotypes. Survival was dependent on expression of RpoS and intact lipopolysaccharide and *Salmonella* serotypes impaired in either one or both of these showed poor survival. Strains lacking SEF17, flagella and the OmpF porin were not affected in their survival characteristics (**FSA B03012**). Other research is investigating the effect of environmental change on the expression of genes involved in infection (**BBSRC 41208**).

6.3.5 The mechanisms of the stress response have been explored at the genetic level. Projects have focussed on identifying genes and proteins which are induced during the bacterial response to stress such as entry into stationary phase (**BBSRC D07416**), nutritional starvation (**BBSRC PRS 12194**) and different nutrient and redox conditions (**BBSRC 0829**) and other environmental factors (**BBSRC 4321208**). Other projects have worked on genes and proteins known to be identified with the stress response in order to understand their function (**BBSRC D05639**, **D10913** and **BFP11355**).

Physiology

6.3.6 A number of projects have been undertaken to investigate *Salmonella* physiology and gene function. These have included the lux-S based quorum sensing system (**BBSRC D17313**) which will have relevance to bacterial infectivity, and work on the role of iron (**BBSRC D11863**), redox active metals

(**BBSRC D19920**), the flavohaemoglobin Hmp (**BBSRC P18939**) and thionate and thiosulphate metabolism (**BBSRC P11074**) all of which are involved in bacterial response to oxygen-related stress.

6.3.7 The interaction between *S.* Typhimurium and amoeba has also been investigated with a view to understanding how the protozoa influence *Salmonella* growth and gene expression in a number of environments (**BBSRC MMI09738/MMI09727, D18179**). This is of relevance in determining aspects of the mechanisms of pathogenesis and survival of *Salmonella*. Similarly, the development of fermenter-based simulations of gut habitats will give information on the interaction of gut commensal organisms with pathogenic bacteria including *Salmonella* (**SEERAD RRI/504/95**).

6.3.8 Understanding the growth of *Salmonella* within the intestinal tract of its host is important. Research includes determining the growth of several *Salmonella* serotypes in calves and pigs, including an assessment of the impact of *Salmonella* pathogenicity island-2 (SPI-2) and the plasmid-borne spv loci on host specificity (**BBSRC 860**). For poultry there is research to understand the energy generation requirements of *Salmonella* serotypes which are able to colonise the alimentary tract of chickens, including considering the impact of different breeding lines of the birds on the bacterial growth (**Defra OZ0320**).

6.3.9 Two projects have looked at the growth of *Salmonella* in food. In one, it was found that the microstructure of food had a significant effect on the survival of a small fraction of bacterial food-borne pathogens, in particular where there are microscopic water-rich niches within the food matrix in which a small number of bacteria were able to survive (**FSA B01001**). Research in project **FSA B03015** considered the growth of *S.* Enteritidis and other *Salmonella* in eggs. It was found that a high concentration of glucose present in the eggs and relatively low iron levels were linked to high levels of bacterial growth. *S.* Enteritidis survived in albumen at hen body temperature, a property was not seen with other *Salmonella* strains that have previously been widespread.

6.4 Gaps in currently funded research

6.4.1 Currently there are many research projects that are exploiting the genetics of *Salmonella* serotypes or are considering the interaction of the bacterium with its environment. There are no clear aspects of the genetics and physiology of the bacterium that are not covered to some extent by existing research. There is a continuing need for research comparing *Salmonella* with other enteric bacteria. A number of genetic and genomic tools have been developed in the last few years, in particular wide range of knock-out mutants. These should be available to the scientific community through conventional mechanisms, generally from the originating researcher. In the funding of future projects, it will be valuable to ensure that these resources are fully exploited, thus avoiding duplication.

6.4.2 There is little research to address the role of phage integration in the epidemiology, survival and pathogenicity of *Salmonella*. This is an area which

could benefit from additional work.

7. PATHOGENESIS AND HOST RESPONSE

7.1.1 Salmonellosis in humans occurs when *Salmonella* penetrate from the gut lumen to the epithelium of the small intestine and then cause inflammation. In animals, disease can occur in a similar manner. In addition, in more extreme cases in humans and animals the bacteria can enter the blood stream and colonize other tissues such as the liver or ovaries.

7.1.2 The majority of research into the pathogenicity of *Salmonella* and the host response to this is undertaken in animals, in particular poultry and other farm animals, as well as mouse models. The research provides the opportunity to explore control and intervention strategies, for example development of vaccines so as to reduce the level of bacterial carriage in poultry and other farm animals. The host-pathogen relationship is an important component of the study of *Salmonella* pathogenesis. A notable feature of this research is the increasing use of host as well as bacterial genomic information and techniques, in particular to explore the host specificity of various *Salmonella* serotypes.

7.2 Overview of current research

The molecular biology of *Salmonella* virulence

7.2.1 There is a significant research effort to apply molecular biology and genomics to the understanding of the virulence of *Salmonella*. Several projects consider the role of *Salmonella* outer membrane proteins (Sops). These proteins are involved in the induction of the host response to infection by the pathogen. Projects have included those investigating the cellular response to individual Sops (**BBSRC D18830**), the structure function and role of selected Sops including the host response (**BBSRC 0177, 0244**), the function of Sop and pathogenicity-island-encoded proteins (Pips) within host cells and the induction of the initial inflammatory response (**BBSRC 0858**), the response of eukaryotic cells to Sop expression (**BBSRC 1035, BBSRC 1116**) and which Sops are specifically involved in gastrointestinal colonisation (**BBSRC BFP11326, BBSRC 756**).

7.2.2 Other proteins, such as *Salmonella* invasion-associated proteins (Sips) have also been identified as being involved in the virulence of the bacterium, and there are projects to determine the role of Sips with respect to Sops (**BBSRC 0219**), and the role of Sips in pathogenesis in pigs (**BBSRC 0177**) and mice (**BBSRC D07439**). A further *Salmonella* surface protein of interest is ShdA: deletion of the gene results in reduced colonisation and the structure:function relationship of the protein is being investigated, including examining the role of the protein in persistence of *Salmonella* in the host intestine (**BBSRC D20084**).

7.2.3 *Salmonella* pathogenicity island (SPI) proteins are also associated with colonisation of the host gastro-intestinal tract. The influence which SPI-4 has

on colonisation and pathogenesis in cattle (**BBSRC 1037**) and SPI-2 in cattle and pigs (**BBSRC 860**) is being investigated.

7.2.4 Research is also being undertaken to investigate the role of the transcription factor SlyA of *S.* Typhimurium (**BBSRC BFP11284**), the *Salmonella* luxS-based quorum sensing system (**BBSRC D17313**), the *Salmonella* pathogenicity island 4 (**BBSRC D19269**) and of lipid A (**BBSRC D09660**) in *Salmonella* virulence and immunity.

7.2.5 There is research which considers the commonality between structures of virulence factors in different species and serotypes of *Salmonella* and enteropathogens generally (**BBSRC JE514316**), applying NMR techniques to the structure:function relationships of the relevant proteins.

Host specificity in pathogenesis

7.2.6 There are several research projects exploring the relationship between virulent, infectious *Salmonella* and the affected host. These include projects to determine the molecular basis of *Salmonella* serotype host specificity (**Defra OZ0319**) and the functional differences in the ability of *Salmonella* strains to invade various enterocyte cell lines (**SEERAD SAC/136/97**). Pathogenicity gene islands which are potentially chicken-specific have been identified and it was also observed that the growth rate of *S. choleraesuis* in pigs is considerably lower than that of *S.* Typhimurium despite the former causing more severe systemic disease (**Defra OZ0319**).

7.2.7 Using signature-tagged mutagenesis (STM), nearly 150 genes have been found which are required for colonisation of the gastro-intestinal tracts of chickens or calves (**Defra VF0101**). Amongst these genes are several possible new pathogenicity islands, at least three of which appear to encode genes required for colonisation of chicks only. Further characterisation of some of these genes has confirmed that they are involved in species-specific colonisation. The use of STM as a mechanism for identifying genes essential for colonisation is now being extended to pigs (**Defra VF0101**).

7.2.8 Successful growth within the host is essential for a pathogen and is dependent on the rate of bacterial replication and the bacterial resistance to killing by the host. The growth of several different *Salmonella* serotypes in pigs and calves is being investigated in particular with a view to understanding the impact of pathogenicity island SPI-2 (**BBSRC S14753**). The presence of this pathogenicity island has been found to influence overall growth of *Salmonella in vivo.* This research contributes to an understanding of the biological basis of strain and serotype:host specificity of *Salmonella*. So does research seeking to identify factors responsible for host-specificity of selected *Salmonella* serotypes (**BBSRC 0302**) and investigating the infection of cattle and pigs with a variety of different wild-type and mutant *Salmonella* serotypes (**BBSRC 10274/0751**) and research into the growth and metabolism of *Salmonella* serotypes capable of colonising the alimentary tract of poultry (**Defra OZ0320**).

Host:bacterial interactions

7.2.9 Developing an understanding of the interaction between the bacterial pathogen and its host is an underlying factor driving much of the research covered in this report. However, there are a number of projects which specifically aim to consider this relationship and to explore its modulation.

7.2.10 Cytokines and other immune system modulators are very important in a host animal's response to infection. For example, project **BBSRC D13683** examined the phagocytic cells involved in locations of infection of *Salmonella*, considering in particular the activation or down-regulation of a variety of cytokine and other immune system molecules. A highly focussed approach is taken by projects **BBSRC 0856** and **BBSRC D14755** where the role of interferon gamma (IFNγ) and IFNγ-producing cells are investigated. In both projects, host cell function and responses are a primary focus of the research. In contrast, research within **BBSRC 4131887** focuses on a variety of aspects of gut immunology, in particular the interaction between lymphocytes and intestinal epithelial cells in order to study host response to infection at a molecular level.

7.2.11 The role of protective cell mediated immunity in the host response to *Salmonella* infection has been examined in mice. The particular focus was on the identification of proteins secreted by the bacteria which were recognised by antibodies and T cells (**BBSRC D11572**).

7.2.12 The mechanism of *Salmonella* infection involves the invasion of individual host cells by the bacteria, and two projects are using fluorescence tagged proteins to investigate bacterial:host interactions at a molecular and cellular level (**BBSRC 41208, BBSRC BBSB02266**)

7.2.13 Mice are used as a model animal for investigating a variety of aspects of pathogen-host interaction. For example, the role of the lipid A domain of lipopolysaccharide (LPS) is critical in causing death in mice infected by *S.* Typhimurium and research is being undertaken to develop further an understanding of how host macrophage responses are affected by the LPS (**BBSRC D16845**). It is suggested, and further research aimed to address this (**BBSRC 9912336**), that this may be in part due to interaction between the LPS and specific Toll-like receptors on the surface of mouse macrophages. The role of lipid A in inducing host response to *S.* Typhimurium infection of mice is also investigated, using a variety of mutants with modified lipid A structures (**BBSRC D09660** and **D09961**). The switching-on of *Salmonella* genes during infection in mice is also being investigated (**BBSRC 41208**).

7.2.14 The mouse typhoid model is used to investigate the role of cytotoxic T-cells in *Salmonella* infections (**BBSRC D04785**) and the function of IL12, both in terms of its influence over bacterial growth and in the development of protective T and B-cell memory (**BBSRC D09737**).

7.2.15 Three projects focus on the immune response seen in chickens following infection with both food-poisoning and potential vaccine serotypes of

Salmonella (**BBSRC BFP11365, 11367** and **0245)**. Further projects focus on the impact of particular *Salmonella* genes on the host immune response to infection. Consistent differences were observed in the duration and excretion of *S. enterica* from different in-bred lines of chickens. However, no differences in serum IgG levels were seen nor in two other genes associated with resistance to salmonellosis in chickens (*SAL1* and *NRAMP1*) (**Defra OZ0314**). Similarly no clear relationship has been found between resistance to caecal carriage of *Salmonella* and expression of a variety of other genes (**Defra OZ0320**). Further work on these issues is also being undertaken in **BBSRC 0753**.

7.2.16 In contrast, there are several projects examining the impact of chicken genes on the response of these hosts to *Salmonella* infection. Approaches include research into genes identified as conferring resistance to *Salmonella* infection (**BBSRC 0237, 0239, 0243** and **GAT09084**). In addition, chicken gene microarrays have been constructed, as well as microarrays for *S.* Typhimurium, in order to compare gene expression profiles during adhesion and invasion of the host cells (**BBSRC 0829**).

7.2.17 The response of different lines of chickens to infection by *Salmonella* spp was found to vary reproducibly. The variation occurred in the mononuclear phagocyte system and macrophages were clearly implicated (**Defra OZ0313**). The cytokine response to invasion by different serotypes was also varied, with *S.* Enteritidis and *S.* Typhimurium inducing a significantly stronger IL-6 response than *S.* Gallinarum, which could provide an explanation for the greater invasiveness of the latter serotype (**Defra OZ0313**).

7.2.18 Pigs from lines resistant and susceptible to salmonellosis have been investigated in order to identify genetic loci known to be implicated in susceptibility in other animals (**BBSRC GAT09015**, **0238**). The *Salmonella* induced intestinal inflammatory response in pigs is also being investigated, particularly considering host specificity of the infective bacteria (**BBSRC D15635, 0923**). Other research shows that *Salmonella* reside within lymph and that the interaction of *Salmonella* with the cells in the intestinal mucosa and mesenteric lymph nodes influences the systemic spread of the bacteria (**Defra OZ0315**).

7.2.19 Completed research found that a possible mechanism of development of resistance to *Salmonella* in gnotobiotic pigs is the substantial infiltration of neutrophils by avirulent *Salmonella* species (**Defra OZ0313**). This work has been extended within an EU Framework IV project (**BBSRC 0246**).

7.2.20 The interaction between *Salmonella* spp and cattle is investigated in **BBSRC 0396, 0755** and **Defra OZ0315**, all of which projects focus on the host animal immune response to bacterial antigens, generally provided as live attenuated strains of *Salmonella* and the consequent expression of cytokines and other immune system modulators by the cattle. Research within **Defra OZ0318** also seeks to understand the role of host (cattle) immunity, levels of excretion and persistence in relation to endemic and epidemic strains of *Salmonella*.

7.2.21 The interaction between *Salmonella* and human intestinal epithelium and immune system is crucial to virulence of the pathogen within humans, and is the subject of study within a number of projects including **BBSRC 41387** and **BBSB01901**.

7.3 Gaps in currently funded research

7.3.1 A wealth of scientific research into the mechanisms of pathogenesis of *Salmonella* has been supported in the last five years by the MSFFG member organisations, and there is extensive research supported internationally in the same field. The most urgent need is to develop a clear understanding of the mechanisms of *Salmonella* pathogenesis and host response as these relate to the real world as opposed to the laboratory. There are a number of recent reviews considering the subject (for example Hensel (2004), Rhen and Dorman (2005)) and further papers in press. However, these do not address the pathogenesis of *Salmonella* or the host response as applied to practical and important everyday issues, including the cause, process and occurrence of food poisoning in humans.

8. ANTIMICROBIAL DRUG RESISTANCE

8.1 The development and spread of antimicrobial resistance, in particular multi-drug resistance, is regarded as a concern in the management of bacterial pathogens in general and *Salmonella* in particular. It is also of concern because of the general issue of increasing antibiotic resistance in many types of bacteria, and the potential for transfer of the genes between bacterial species. As a reflection of the scale of the issue, over half of the *Salmonella* isolates from a study of chicken carcasses for retail sale were found to be resistant to at least one antimicrobial drug and multiple drug resistance was found in 23% of the isolates (**FSA B18002**).

8.2 Overview of current research

8.2.1 A GyrA mutation assay was developed for rapid detection of quinolone antibiotic resistance, and has been used in a number of investigations of major outbreaks of quinolone-resistant *S.* Typhimurium (**FSA B10001**). Work was also undertaken to develop methods for detection of the ACSSpSuT[18] resistance island leading to the identification of the resistance gene island in other *S.* Typhimurium phage types and *Salmonella* serotyopes (**FSA B10001**). Genomic methods are also being established in order to further characterise resistant strains, and techniques such as MLST and PFGE are being used. Using PFGE it was found that 41 distinct pulsed field profiles within a single multi-resistant strain of *S.* Typhimurium could be identified. This technique was the most powerful subtyping method among those investigated (**FSA B01013)**, including DNA gyrase mutation analysis which was only applicable

[18] Ampicillin, chloramphenicol, streptomycin/spectinomycin, sulphonamides and tetracycline resistance island

to strains exhibiting resistance to quinolones.

8.2.2 There are a number of possible routes for the spread of antimicrobial resistance in farm animals. These include the on-farm animal management, and it was noted that there were higher levels of contamination of pigs with multiple resistant *S.* Typhimurium in continuously occupied or poorly disinfected houses (**Defra OZ0134**).

8.2.3 The possibility of transferring antimicrobial resistance factors through the spreading of farm wastes is addressed in **Defra OD2005, OD2008**. Transfer of antibiotic resistance was not detected in stored and spread manure (**Defra OD2005**). There was, however, some evidence that animal manure, when applied to pasture in the summer, could provide a favourable environment for the exchange of genetic material between bacteria, and this might facilitate the spread of antimicrobial resistance (**Defra OD2008**).

8.2.4 The use of antimicrobial growth promoters in animal feed has been significantly curtailed by EU legislation[19]. However, there remains a need to understand how their use influences the development of antimicrobial resistant bacteria. The evolutionary pressure on *Salmonella* in chickens under such circumstances is being investigated (**BBSRC 0772**) and a generic model for investigating the development of antimicrobial resistance has been established (**Defra VM02105**). There has been a particular focus on flouroquinolone antibiotics, against which the principal mechanism of resistance in *Salmonella* is mutations in the gyrA gene (**Defra OZ0502, VM02100**). It was found that flouroquinolone resistance did develop in both pigs and chickens, and that the use of specific antibiotics coupled with specific disinfectants might accelerate the occurrence of multiple antibiotic resistant mutants of *Salmonella* (**Defra OD2004, VM02100**). The mechanisms of antibiotic resistance are also considered in **Defra OZ0132**.

8.2.5 Given the change in farm practice away from use of antimicrobial growth promoters, there is a need to determine what effect the withdrawal of these factors will have. This is being studied by investigating the persistence of antimicrobial resistance for up to 12 months after withdrawal of the feed additives from both pigs and chickens (**Defra VM0292**).

8.2.6 Recent projects have been funded to investigate the use of non-antibiotic antimicrobial agents (**Defra OD2010**) and to determine concentrations of flouroquinolones which could be used with poultry and not lead to the development of antimicrobial resistance (**Defra VM02201**).

8.3 Gaps in currently funded research

8.3.1 With the changes in on-farm practices and increased awareness of the risks of antimicrobial resistance, the focus of future research in this area could be the development of antimicrobial resistance in bacterial populations in response to changes in treatment and management regimes. Research on the

[19] http://europa.eu.int/eur-lex/pri/en/oj/dat/2003/l_268/l_26820031018en00290043.pdf

effectiveness of alternatives to antimicrobial agents may be also be appropriate, as may investigation of the spread of antimicrobial resistance in integrated poultry and pig breeding and production networks.

8.3.2 The MSFFG intends to produce a research overview paper on antimicrobial resistance in the future.

9. EPIDEMIOLOGY, REDUCTION AND ELIMINATION, RISK IDENTIFICATION AND MANAGEMENT: RESEARCH SPECIFIC TO *SALMONELLA* IN POULTRY

9.1 In contrast with humans and other animals, there is the potential to eliminate *Salmonella* from UK-produced, intensively-housed poultry within the food industry. This is a reflection of the importance of vertical transmission of *Salmonella* in poultry, coupled with the structure of the poultry and egg producing industry which is in effect a pyramid. The breeding of poultry for either meat or egg production is restricted to a small population of birds, which are *Salmonella*-free. The numbers of these birds is expanded through several stages (breeder, hatchery, layer or broiler flock) and then the products are moved on to the processor and thence to the retailer or caterer and finally to the consumer. *Salmonella* control measures can be implemented at each of the steps and processes involved. Thus it is conceivable that the bacterium could be eliminated at least from parts of the chicken production sector, but it is unlikely that this could be achieved across all the various different practices and scales of poultry farming operation within the UK. However, there has been considerable progress in this general area in the last ten to fifteen years and substantial control of *Salmonella* within the poultry production sector is currently feasible. This is not yet the case with cattle or pig production (See Section 10).

9.2 There has also been considerable concern about the occurrence of *Salmonella* in eggs, leading to the FSA instigating its Poultry and Egg Research Programmes[20]. Again, research in this area has led to greater understanding of the issues.

9.3 Overview of current research

Epidemiology and occurrence

9.3.1 Work in Northern Ireland to determine the occurrence of *Salmonella* found that the bacterium could be isolated from 25% of egg-laying poultry flocks (**DARD 9904**). There are no comparable data for Great Britain, but a Europe-wide study of the issue is nearing completion and will give information on country-wide infection levels.

9.3.2 Research into the transmission of *Salmonella* between egg-laying poultry flocks (**Defra OZ0321**) and from hatching to slaughter for broiler flocks (**FSA B03008**) is being undertaken. The latter project examined 8,400 poultry

[20] http://www.food.gov.uk/science/research/researchinfo/foodborneillness/eggsresearch/b15review

samples for *Salmonella* but did not find any on farms. Examination of the water delivery systems and drinking water for poultry did not find any *Salmonella* in the samples taken, although significant levels of *Campylobacter* were observed (**FSA B03010**).

9.3.3 The occurrence of *Salmonella* in chicken and other foods for retail sale has been investigated. An initial project looked at the presence of *Salmonella* on retail chicken in England (**FSA B08008**) and found *Salmonella* serotypes present on 25% of carcasses and on 19% of samples representing the inside and outside of the carcass packaging (Jørgensen *et al* (2002)). In the later FSA national survey of retail chicken, it was found that the overall level of frequency of contamination of retail chicken in the UK with *Salmonella* was 5.7%. Significantly, fresh chicken samples showed a lower frequency of contamination (4.0%) than frozen (10.4%). It was also noted that UK produced chicken showed a lower frequency of contamination with *Salmonella* (8.3%) than imported (13.6%), but this was due to the fact that the majority of the latter samples were frozen. Of the 30 different serotypes isolated, *S.* Typhimurium was the most frequent, accounting for 14% of the isolates, all but one from UK-produced chicken. Other serotypes isolated included *S.* Heidelberg*, S.* Infantis*, S.* Ohio and *S.* Thompson*.*

9.3.4 The occurrence of *Salmonella* in UK-produced eggs on retail sale has also been investigated (**FSA B18007**). Samples of eggs were purchased throughout the United Kingdom and pooled samples of 6 eggs each were assessed for the presence of *Salmonella* on or in the eggs. It was found that 0.34% of the samples were contaminated with *Salmonella*. Although a direct comparison cannot be made with the previous study, which was held in 1995/6 in England only, by inference there has been a three-fold reduction in the levels of *Salmonella* contamination since that survey, reflecting some of the measures introduced into the egg and poultry industry to control the occurrence of the pathogen.

9.3.5 The occurrence of *Salmonella* in egg-producer farms has been assessed (**Defra OZ0317**) and this study is now being extended (**Defra OZ0321**). *S.* Enteritidis was the principal serotype occurring on these farms, with other serotypes being uncommon (in contrast with broiler farms). The general findings included that prevalence was lower in farms which used vaccination, although not always; that cleaning and disinfection measures were often insufficiently thorough to reduce contamination; that there were pools of persistent carriage in flies and rodents; and that persistence of *S.* Enteritidis for many years was usual in problem cage flocks. However, barn and free-range flocks usually became *Salmonella*-free within 1-2 flock cycles. It was also noted that even vaccinated flocks produced some eggs which were contaminated (internally and externally) and that a low level of contamination did spread from eggs to packaging equipment and hence to other eggs. It was demonstrated that washing of the eggs with ionised water did reduce surface contamination, whereas disinfection of packaging plants did not.

Reduction and elimination: vaccine development

9.3.6 There already exist a number of commercially available vaccines against *S.* Enteritidis and *S.* Typhimurium for use with poultry[21] and their use is regarded as an additional measure to increase the resistance of birds to *Salmonella* and to reduce the levels of shedding of the bacteria.

9.3.7 Research on the mechanisms of immunity in chickens in response to live attenuated vaccines is addressed in **BBSRC 245, 246.** In addition, the mechanisms of immunity to both food-poisoning and potential vaccine serotypes of *Salmonella* in chickens are being examined with a view to developing improved live vaccines (**BBSRC BFP11365, BFP11367**).

Reduction and elimination in the food-supply chain

9.3.8 As poultry production is the perceived principal source of *Salmonella* in the food-supply chain, there is significant research effort to identify ways of reducing the levels of the bacteria at various stages of the supply chain. Critical examination of the process for rearing chickens for meat found that there was considerable variation in practices for sampling and testing (**FSA M01017**) and monitoring and control of *Salmonella* across the industry (**FSA ZB00023, ZB00034**) and suggested that improved practices could be identified. Further work showed that a critical point of contamination in broiler production was the feed mills (**FSA B03001**) followed by the hatchery. Advice on management of the feed manufacture and the egg preparation in order to reduce the levels of *Salmonella* has been developed based on results from these projects and used in the industry.

9.3.9 It was also found that systems for washing poultry transport crates were not adequate and improved methodologies, including adequate disinfection by immersion, would be needed (**FSA ZB00033**).

9.3.10 Levels of enteric bacteria on raw poultry can be reduced by a number of treatments such as steam, hot water, rapid hot and cold air-drying and surface freezing (**FSA M01019**) and commercial systems are being developed based on the cold-air drying approach. For storage of carcasses, research showed that a mixture of *S.* Enteritidis and *S.* Typhimurium was not able to grow over a 15-day period on chicken held at temperatures at or below 6°C (**FSA B12004**).

9.3.11 The production of eggs for sale is also a route by which *Salmonella* can be introduced to humans. It has been found that washing eggs can lead to a reduction in bacterial levels, but only if appropriate methodologies are used (**FSA B03017**). The general conclusion was that egg washing is on balance not valuable.

[21] Eg see http://www.intervet.co.uk/Species_Pages/Poultry/Poultry_Vaccines.asp?ldt=2&idxContentType=&fromCache=1&page=2

Risk identification and management

9.3.12 Risk assessment models for *Salmonella* in poultry have been developed (**FSA B03006**). Research is also being undertaken to develop improved approaches to risk assessment for food-borne pathogens including *Salmonella* in poultry (**BBSRC 0455**). Analysis of research findings and information from leading UK poultry producers led to the conclusion that effective controls for *Salmonella* risk management were in place within the industry, although not for *Campylobacter* (**FSA B03005/6/7**).

9.3.13 Eggs are generally seen as an important source of *Salmonella* infection for humans. Research found that the levels of *Salmonella* on the surface of eggs declined with time but that it was never possible to state that there were no *Salmonella* present (**FSA B03016**). In addition, it was found that there was a relatively high transfer from the shell to the egg contents and hands on breaking of the egg.

9.3.14 An evaluation of the US *Salmonella* Enteritidis risk assessment model has been undertaken (**FSA B01017**). It was concluded that although the methodologies used in developing the model were appropriate, the model as it stood would not provide the most effective analysis of UK egg production, sale and use. A simplified model, derived from the US one, did provide a starting point for the development of a model(s) for use in the UK. The FSPB has funded a project (**03-RESR-005**) to develop a risk assessment model for *Salmonella* in shell and processed eggs on the island of Ireland that will investigate the effect of vaccination on laying flocks.

9.3.15 Understanding the impact and risks associated with consumer behaviour in relation to handling and cooking chicken meat and eggs as well as other kitchen practices, is important. It was found that a significant number of consumers followed practices (such as how meat was stored, washing of boards and surfaces, use of cloths) that were likely to lead to spreading of bacteria within the kitchen (**FSA B02016**). It was noted that *Salmonella* was not found in kitchen cloths, although other enteric bacteria, including *Escherichia coli* were (**FSA B02015**). In a different study (**FSA B01015**) *Salmonella* was generally not detected in chicken or on kitchen surfaces. It was also noted that there was little occurrence of *Salmonella* on the packaging materials of raw chicken nor on shelves where raw, packed chickens were kept within retail outlets. This was seen as indicating that cross-contamination from the packaging was not a significant source of risk (**FSA B03002**).

9.3.16 The possible transfer of *Salmonella* from contaminated chicken carcasses in the kitchen was analysed (**FSA B02010**). Although little contamination occurred, there were frequent hygiene errors which could lead to cross-contamination. The cooking of chicken in both the domestic context and catering establishments was generally found to be adequate by standard industry tests (**FSA B01015**) but the potential remained for cross-contamination in kitchens.

9.4 Gaps in currently funded research

9.4.1 The progress in understanding of *Salmonella* in poultry has been substantial in recent years, with a noticeable benefit in reducing the incidence of the bacterium within both the chicken meat and egg industries. There is however, less understanding of the same issues in other poultry meat production notably the turkey breeding and meat production industry.

9.4.2 There also remain the issues associated with persistence of *S*. Enteritidis in some farms and commercial cage layer houses, which continues despite vaccination. There may be a need for further research on these problems, as well as on the transfer of *Salmonella* from feedmills, hatcheries and persistently infected farms.

9.4.3 A further area where some additional research could be beneficial is in developing a greater understanding of consumer practice.

10. EPIDEMIOLOGY, REDUCTION AND ELIMINATION, RISK IDENTIFICATION AND MANAGEMENT: RESEARCH RELATING TO NON-POULTRY MEAT

10.1 Poultry meat and eggs are the most important sources of *Salmonella* for the human food-supply chain, and therefore have been a major focus of research in recent years. However, *Salmonella* can also be introduced to man from red meat, and research with farm animals other than poultry is therefore supported.

10.2 Overview of current research

Epidemiology and occurrence

10.2.1 There are both endemic and epidemic strains of *Salmonella*. Understanding the relationship between these and their host organisms is important for development of control strategies for the bacterium. Research to investigate and model endemic and epidemic *Salmonella* in pigs and cattle, as well as exploring herd immunity and excretion differences in cattle, is being undertaken within project **Defra OZ0318**.

10.2.2 It was found that in England and Wales up to 25% of dairy herds were positive for *Salmonella* (generally with no clinical symptoms), but that the proportion of herds carrying strains commonly associated with human illness was low (**Defra OZ0135**). It was noted in this study that there appeared to be a decline in antimicrobial drug resistant strains. In a similar study in pigs, a survey of 161 farms found that 70% were infected with *Salmonella* (**Defra OZ0134**) and that there was persistence of multiple antibiotic resistance in *S.* Typhimurium for at least two years before a reduction commenced in some farms due to reduced stocking levels. Both projects noted that interventions and control measures on farms were generally not successful as they were not applied sufficiently rigorously. Further work to estimate the occurrence of *Salmonella* in pig herds in the UK and to develop appropriate control strategies has been

started (**Defra OZ0316**). A study of *Salmonella* in pigs for slaughter is also being undertaken.

10.2.3 Other on-farm epidemiological studies with *Salmonella* involve investigation of the transmission of food-borne pathogens from the farm to the abattoir (**Defra VF0201**).

10.2.4 The occurrence of *Salmonella* on animals arriving at abattoirs and on carcasses has been investigated in a number of projects. It was found that *Salmonella* was not found on sheep carcasses, nor in bedding or lorry samples (**FSA M01011**). In a study of meat plants, 2 out of 13 plants had lamb carcasses which tested positive for *Salmonella*, whilst 4 out of 17 and 4 out of 16 plants had cattle and pig carcasses respectively testing positive for *Salmonella* (**FSA M01014**). A similar result for pig carcasses contaminated with *Salmonella* (2 carcasses out of 10 examined) was found in a study which also investigated whether carcasses could be treated so as to reduce contamination (**FSA M01002**). This latter approach was found to be effective but it was noted (**Defra OZ0134**) that subsequent handling of carcasses (in particular, evisceration) was likely to increase the contaminant level again.

10.2.5 In Northern Ireland, it was found that 6 out of 210 faecal samples from beef carcases taken at abattoirs contained *Salmonella* (**DANI 9723**). Work is also being carried out to determine the likely extent of contamination of raw milk with a variety of pathogens, including *Salmonella* (**FSPB 00-RESR-046**).

10.2.6 The levels of *Salmonella* in abattoir wastes were investigated, but (unexpectedly) none was detected (**FSA B05008**).

Reduction and elimination: vaccine development

10.2.7 A number of projects are seeking to develop, or contribute to the development of, potential vaccines for farm animals. This is on the basis that vaccinated animals would neither be carriers nor fall ill from *Salmonella* infections. The clear advantage would be a reduction in the load of *Salmonella* introduced into the food-supply chain and therefore reaching humans. Approaches have included evaluating the immune response of cattle to *Salmonella* antigens (**BBSRC 0396, D11217**) and to live attenuated *Salmonella* vaccines (**BBSRC 0755**). The antigenic properties of around 100 identified proteins are being tested in mice to determine both the impact of host background and the potential of antigens as vaccine components (**BBSRC D20030**). Mouse models are being used to investigate the role of cell-mediated immunity in the effect of live *Salmonella* vaccines (**BBSRC D04785**).

10.2.8 Other projects could lead to the creation of attenuated live *Salmonella* strains based on studies of genes required for pathogenesis (eg **Defra OZ0319, BBSRC APG19114**) or to multi-component vaccines for cattle (**BBSRC 756**). The SPI-2 gene has been identified as a possible locus that could be exploited in developing a live attenuated vaccine for large domestic animals (**Defra OZ0315**).

Reduction in the food supply chain: farms and farming practice

10.2.9 Reduction of the levels of *Salmonella* in the farm is regarded as important, and there is research to investigate farming practices which may affect the spread of human bacterial pathogens through farm animal populations. Studies include sheep farming, transport and marketing (**FSA M01015**) and an investigation of the constraints to uptake of biosecurity measures with both cattle and sheep (**Defra OZ0144**).

10.2.10 Specific issues which are being addressed include the significance of organic waste storage and application (**FSA B05003, B17002, Defra WA0656, WA0804**). It was found that the common agricultural practice of spreading manure onto farmland may be a contributory factor in the occurrence of food-borne disease in the UK (**FSA B05003**). Results from the project are currently being used to support the writing of guidelines on manure management on farms. Research to consider the impact of adopting recommended approaches to manure management and pathogen control recognised that there were significant practical and cost implications, although it was possible to develop appropriate best practice for manure management (**Defra WA0656**).

10.2.11 There is a need to be aware of the occurrence of *Salmonella* in animal feeds and an evaluation of this source of infection is in progress (**Defra OZ0711**). It may be possible to develop feeds which discourage the growth of bacterial pathogens (**BBSRC 15003104**).

10.2.12 The cleanliness of cattle at the point of slaughter is of importance, and the FSA has commissioned research to examine whether recent practices are beneficial or not (**FSA M01013**). Output from this project is being used to assist farmers in development of best practice, as is output from research into the spread of *Salmonella* and other bacteria between cattle in on-farm, abattoir and market environments (**FSA M01009**).

Risk identification and management

10.2.13 The risks associated with different components of on-farm practice have been studied in a number of research projects. Early work to assess the risks to food of spreading organic farm and abattoir waste found that significant additional research was required on pathogen decay rates in slurries and manures (**FSA B17002**) and this was addressed in other research projects (eg **FSA B05003**). The potential risks associated with the different routes by which pathogens may be transferred from the farm to the wider environment are assessed in **Defra WA0804**, including consideration of the role of ground water and water supplies.

10.2.14 Analysis of the risks associated with pork production noted that there was possibly a greater risk of *Salmonella* contamination than was generally recognised (**FSA M01002**).

10.2.15 It was noted that a variety of different techniques, generally traditional, were being used in the food industry for measuring contamination by *Salmonella* and other food-borne pathogens. In addition, there are significant barriers to the introduction of new and initially more expensive technologies which may be a future issue (**FSA B09005**).

10.2.16 In tests to consider the effectiveness of cooking sausages contaminated with *Salmonella*, it was found that certain cooking methods, in particular those where the sausages were cooked over a high heat for a relatively short period of time, were not sufficient to destroy the pathogen, despite the sausages appearing cooked (**FSA B02013**).

10.3 Gaps in currently funded research

10.3.1 In the 1999 *Salmonella* report, it was noted that there was a need for more epidemiological data on *Salmonella* and salmonellosis in humans, including outbreaks. There is ongoing surveillance, which is addressed by the Epidemiology of Foodborne Infections Group and the Surveillance Group for Diseases and Infections in Animals[22]. One aspect where there could be benefit from further research is the epidemiology of food-borne *Salmonella* in humans.

10.3.2 There may be a need for more research to examine the patterns of occurrence of *Salmonella* in farm animals (including the effect of the movement of live cattle and sheep for trade) in relation to infections and outbreaks in humans. There is a need to clarify whether the introduction of older beef to the food-supply chain will have an impact on the levels of food-borne pathogens, including *Salmonella*. Information from the United States indicates that cull cows are more likely to be infected with *Salmonella* than one- to two-year old cattle at slaughter, although it should be noted that feed and transport regimes in the US are significantly different from those in the UK. It is also noted in several projects that there may be a significantly higher occurrence of *Salmonella* in farm animals than is being reported, and there may be benefit in research to determine whether this is the case.

10.3.3 With respect to the reduction and elimination of *Salmonella* from the farm and food-supply chain, many of the issues for further research identified in the 1999 *Salmonella* report have been addressed. Significant research has been done towards offering best practice guidelines, for example the Zoonoses Action Plan (ZAP) that has been developed for Quality Assured pig production[23]. Perhaps the principal area where further research would be beneficial is in the area of vaccination as a route to reducing levels of *Salmonella* in farm animals. There would also be value in testing lymphoid tissue in pigs, particularly mesenteric lymph nodes, for *Salmonella* in both the study of the persistence of infection and also as a measure of risk for animals entering the abattoir.

[22] http://www.defra.gov.uk/animalh/diseases/control/sgdia/
[23] http://www.bpex.org/technical/zap/default.asp

10.3.4 In order to develop a suitable vaccine, it is necessary to test a variety of different approaches. Research is needed to determine whether the use of vaccines would be effective in reducing the levels of *Salmonella* in farm animals, and in turn whether there would be a reduction in the levels of *Salmonella* in the food-supply chain. Current vaccines are not effective if *Salmonella* is already present on the farm, and better vaccines, as well as greater understanding of how best to use current vaccines, are needed to address this. Some knowledge of whether the frequent use of vaccines in poultry and other farm animals would have a (possibly undesirable) effect on consumer practice could also be valuable.

10.3.5 Finally, there continues to be a need for surveillance and quantification of the occurrence of *Salmonella* in key stages of food production.

11. REDUCTION AND ELIMINATION: RESEARCH RELEVANT TO FOOD PRODUCTION IN GENERAL

Reduction in the food supply chain: disinfectants and cleaning

11.1 In research on the occurrence and management of biofilms it was concluded that approximately 28% of food contact surfaces and 58% of environmental surfaces (including floors) had high numbers of bacteria present, which was taken as evidence for the presence of biofilms (**FSA B01016**). The bacterial populations were found to include very few organisms associated with food-borne disease and no *Salmonella*, *E. coli* O157 or *Campylobacter* were isolated. Cleaning did not have a significant effect on the levels of bacteria, and there was some evidence from a preliminary study that biocide resistance could develop.

11.2 The prevention of the formation of biofilms was considered in project **FSA B01006**. In this research the adherence of food-borne pathogens including *S.* Typhimurium to polymers which could have both a hydrated state and a collapsed hydrophobic state was investigated. It was concluded that at least some bacteria (for example *L. monocytogenes*) were less able to colonise the polymers when in the hydrophobic state. This was seen as offering possibilities in the development of coatings for surfaces in the food industry which could reduce the adhesion of bacteria and subsequent formation of biofilms.

11.3 A limited amount of research has been undertaken to investigate the applicability of different disinfectants and cleaning regimes on the reduction of the levels of *Salmonella* in the food supply chain. A wide range of disinfectant products has been found to be used in the food industry to combat food-borne pathogens (**Defra FS3206**). However, novel approaches are still required and one such route was the application of dry ice pellets to various surfaces used in the industry (**FSA B02006**). This was found to be effective at destroying *S.* Enteritidis amongst other pathogens on food grade surfaces, but when applied to meat and carcasses the treatment caused extensive destruction of the tissues.

11.4 To support the understanding of physiological stress experienced by *S.* Typhimurium during food processing and help in the identification of points within the food supply chain where there are increased risks of transfer of infection, work has been done to identify protein markers of stress caused by exposure to low temperature or hypochlorite. Although no suitable stress-specific marker proteins were found for cells exposed to hypochlorite, initial evidence was found for a suitable marker for use in an assay to identify cells which had been exposed to low temperatures (**FSA B01008**). In addition, efforts are being made in conjunction with other funders[24] to bring together all the data on food-borne pathogenic bacterial response to stresses in the food-supply chain (**FSA B01012**). This collaboration has led to the establishment of a public database of growth, survival and inactivation data for bacteria in diverse environments of relevance to food processing operations[25].

11.5 Novel approaches to cleaning of raw, ready-to-eat fruit and vegetables have also been sought (**FSA B02005**). Ultrasound and photodynamic washing techniques were examined, but it was found that neither was likely to be an improvement on existing techniques.

11.6 Gaps in currently funded research

11.6.1 Good hygiene at all stages in the food-supply industry is of real importance, and there will always be a need to assess the utility and efficacy of novel disinfectants, and to establish the most appropriate protocols for use of any selected materials and processes, whether new or established. However, there is unlikely to be a need for specific research in this area.

12. OTHER

12.1 Three projects funded by MSFFG members have considered the possible long-term sequelae of *Salmonella* infection in humans. **BBSRC AU00** includes investigation of *Salmonella* induced arthritis in mice, with further work on reactive arthritis being addressed in **BBSRC AU/1/99**. In addition, an archive for DNA and other samples from gastrointestinal pathogens has been established and will be used in the analysis of data from the Infectious Intestinal Disease studies (**FSA B14004**).

[24] The funders and supporters are the Food Standards Agency and the Institute of Food Research, UK, and the USDA Agricultural Research Service and its Eastern Regional Research Centre, USA

[25] http://www.combase.cc/ http://wyndmoor.arserrc.gov/combase/

13. CONCLUSIONS

13.1 The work described in this report demonstrates that during recent years there has been a substantial amount of research on *Salmonella* in the UK. It is notable that the level of effort has been higher with *Salmonella* than for either *Campylobacter* or *E. coli* in the same period[26].

13.2 As a result, there has been considerable advance in the understanding of the molecular biology of *Salmonella*, in particular with respect to the role of specific genes in the pathogenesis of the bacterium and in host-pathogen interactions. There is, however, a danger of there being a gap between this understanding, developed primarily through laboratory-based studies and its application to real-life situations "in the field". Ensuring that there is a link between laboratory and "field" research has relevance in a variety of applications, in particular in the development of novel vaccines, the understanding of what happens in the host when infected with a pathogen and how new types and strains of food-borne pathogens emerge. This latter point is important as, for example, there has recently been a decline in the occurrence of one particular phage type of *S.* Enteritidis, PT4, relative to other phage types of *S.* Enteritidis[27].

13.3 One area of research has, in particular, made very substantial advances to the benefit of public health. Since the mid-1990s there has been a major effort, addressed on a number of fronts, to understand the issues of chicken meat and egg-associated *Salmonella* infections in humans. The research effort has been aimed at many aspects of poultry production, as well as retail, catering and consumer practice. As a consequence, this area of *Salmonella* research is likely to be less active in the near future, other than in the development of increased understanding of the interaction between *Salmonella* serotypes and the host birds. There may also be a need for research to explore the applicability of knowledge derived from research on chickens to other types of poultry meat for example turkey, and to develop an understanding of why there remain some persistent problems within the poultry industry despite the general and significant improvements. Benefits may also accrue by extending the experience with chicken to other farm animals, in particular pigs.

13.4 The success in reducing the levels of *Salmonella* in the poultry industry, and of associated salmonellosis in humans, has been considerable. There has also been real progress in understanding the issues of reducing *Salmonella* levels in other components of the human food-supply chain. The development of vaccines and other effective interventions needs ongoing research, but this should be in the context not only of laboratory studies but of real-life application, including acceptability to and take-up by the production industry.

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[26] See http://www.foodstandards.gov.uk/science/research2/research_archive/pubfund/microbiosafe/vtec http://www.food.gov.uk/science/research/researchinfo/food-borneillness/microfunders/campylobacter for evidence

[27] http://www.hpa.org.uk/infections/topics_az/salmonella/data_human_se.htm

13.5 The relationship between humans and food-borne pathogens is always of importance, but it can be a difficult area for research. However, the many advances which have been made in understanding of *Salmonella* would be enhanced by further work with humans, including disease studies, epidemiology and work to understand consumer practice. There would also be benefit in more intensive molecular epidemiological comparisons of important *Salmonella* serotypes from the main sources of the bacterium, including livestock, foods, imported foods and humans. These and other studies could contribute to gathering more quantitative *Salmonella* data in order to fill the gaps in farm-to-fork risk assessments.

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GLOSSARY

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

Biofilm
A thin layer of living cells, usually micro-organisms, coating a surface.

Commensal organisms
Two or more organisms which live together and share food resources, one species benefiting from the association and not harming the other.

Cytokine
A protein or polypeptide produced by a cell which affects the growth and differentiation of that or another cell.

Dynabead®
A commercially-available magnetic microbead to which specific antibodies are attached. These recognise and bind an antigen, thus allowing separation of the antigen (and any organism it is on the surface of) from other components.

ELISA
Enzyme-Linked Immunosorbant Assay. A technique which uses enzyme reactions as indicators. The sandwich assay is a double-layer procedure and visualises specific antibody. The antigen is sandwiched between the antibody and the secondary labelled antibody.

Endemic
Present at relatively low levels in the population all the time.

Epidemic
An outbreak of a disease affecting a large number of people at the same time.

Gnotobiotic
A completely closed biological environment in which all organisms are known.

Lipid A
The glycolipid component of bacterial lipopolysaccharide.

LPS
See lipopopolysaccharide.

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

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

PCR
The Polymerase Chain Reaction. A widely-used technique to generate multiple copies of a target DNA sequence by amplification.

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

Pip
Pathogenicity-island-encoded protein.

RFLP
Restriction Fragment Length Polymorphisms. Variation in DNA sequence that is easily recognized because it occurs at a site where a restriction enzyme cuts a specific sequence, producing DNA fragments of varying lengths. RFLPs often serve as genetic markers and are used to distinguish between different subtypes of bacteria.

Serotypes
Subdivisions of a (bacterial) species identified by their antigenic characteristics.

Serovar
See serotypes.

Sip
Salmonella invasion-associated protein.

Sop
Salmonella outer membrane protein.

Spv locus
Salmonella plasmid virulence genes.

STM
Signature Tagged Mutagenesis provides a means of identifying virulence genes in bacteria which are essential for the process of infection in a chosen animal model.
http://www.microscience.com/stm.pdf .

VTEC
Verocytotoxin-producing *Escherichia coli* that characteristically produce powerful toxins that kill a variety of cell types, including Vero cells on which their effects were first demonstrated.

APPENDIX 1: DATA FOR MORBIDITY AND MORTALITY OF *SALMONELLA*

Table 1: Indigenous Food-borne Disease in England and Wales, 2000 (Adak *et al* (2002))

					Indigenous food-borne disease (excluding infection due to travel etc)				
	All Laboratory reports	**Organism as % of bacterial laboratory reports**	**Organism as % of all laboratory reports**	**Estimated cases***	**Organism as % of estimated bacterial cases**	**Organism as % of estimated all pathogen reports**	**Deaths**	**Organism as % of bacterial deaths**	**Organism as % of all deaths**
Campylobacter	55888	63%	48%	359466	58%	26%	86	22%	18%
VTEC	896	1%	0.8%	995	0.2%	0.07%	22	5.5%	4.6%
Salmonella	15036	17%	13%	41616	7%	3%	119	30%	25%
Listeria monocytogenes	98	0.1%	0.08%	194	0.03%	0.01%	68	17%	14%
All bacteria	88650		76%	607950		44%	395		82%
All viruses	18718		16%	84051		6%	17		3.5%
All parasites	9222		8%	4728		0.3%	3		1%
Unknown agents	Not applicable			642043		46%	65		13.5%
All pathogens	116590			1388772			480		

* For estimation methods, see Adak *et al* (2002)

Salmonella-caused deaths as % of laboratory reports of *Salmonella*-caused food-borne disease: 0.8%
Salmonella-caused deaths as % of estimated cases of *Salmonella*-caused food-borne disease: 0.3%

Table 2: Estimated food-borne illnesses and deaths caused by known pathogens, United States (Mead *et al* (1999))

	Estimated cases*	Organism as % of estimated bacterial cases	Organism as % of all pathogen cases	Deaths	Organism as % of all bacterial deaths	Organism as % of all deaths
Campylobacter	1,963,141	47%	14%	99	8%	5%
***E. coli* O157:H7**	62458	1.5%	0.5%	52	4%	3%
Salmonella* *	1,341,873	32%	10%	553	43%	31%
Listeria monocytogenes	2493	0.06%	0.02%	499	38%	28%
All bacteria	4175565		30%	1297		72%
All viruses	9282170		67%	129		7%
Al parasites	357190		3%	383		21%
All pathogens	13,814,924			1809		

* For estimation methods, see Mead *et al* (1999). Note that the data are taken from Table 3 in the paper.

** Excluding *S.typhi*

Salmonella-caused deaths as % of estimated cases of *Salmonella*-caused food-borne disease : 0.04%

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

Project Code	Title	Funder	Institution	Start Date	End Date
APG19114 APG19115 APG19116	A novel genome-wide evaluation of mutants in *Salmonella enterica* serovar Typhimurium: application to drug and vaccine development	BBSRC	University of Cambridge, University of Newcastle-Upon-Tyne, University of Oxford	Jun-2003	Jun-2006
AU/1/99	Regulation of mucosal immune responses and induction of autoimmune arthritis	BBSRC[28]	Edward Jenner Institute for Vaccine Research	Mar-2000	Nov-2003
AU00	Autoimmunity Group	BBSRC	Edward Jenner Institute for Vaccine Research	Nov-1995	Oct-2005
BBSB01901	Analysis of *Salmonella* infection of intestinal M cells	BBSRC	University of Bristol	Jun-2004	Jun-2007
BBSB02266	Interactions between host and pathogen at the single cell level *in vivo*: dynamics and determinants of bacterial growth and distribution during *Salmonella* infection	BBSRC	University of Cambridge	Apr-2005	Mar-2008
BFP11284	Characterisation of the *Salmonella* Typhimurium transcription factor and virulence determinant SlyA	BBSRC	University of Sheffield	Jun-1999	Jun-2002
BFP11326	Secreted effector proteins of *Salmonella*: structure, functions and their role in virulence	BBSRC	Institute for Animal Health	Sep-1999	Sep-2002
BFP11355	Role of the cpx regulon in *S. typhimurium* physiology and virulence	BBSRC	University of Glasgow	Apr-1999	Apr-2003
BFP11365	Mechanisms of immunity to food-poisoning and vaccine serotypes of *Salmonella* in chickens	BBSRC	University of Cambridge	Oct-1999	Oct-2004

[28] Edward Jenner Institute for Vaccine Research is supported by Department of Health, Medical Research Council, BBSRC and GlaxoSmithKline

BFP11367	Mechanisms of immunity to food-poisoning and vaccine serotypes of *Salmonella* in chickens	BBSRC	Institute for Animal Health	Sep-1999	Sep-2004
D04785	Cell mediated immune mechanisms in salmonellosis	BBSRC	University of Newcastle-Upon-Tyne	Jan-1997	Oct-2001
D05639	Role of sigma factor E regulated genes in *Salmonella* pathogenesis and immunity	BBSRC	University of Glasgow	Nov-1996	Nov-1999
D06412	Infectivity and physiology of non-culturable cells of *Salmonella* Typhimurium	BBSRC	University of Newcastle-Upon-Tyne	Aug-1996	Mar-2000
D07416	Comprehensive analysis of stationary phase survival regulons of *Salmonella*	BBSRC	University of Southampton	Aug-1997	Aug-2000
D07439	The role of Secreted proteins (sips) and their interactions with host cells in virulence of *Salmonella*	BBSRC	Institute for Animal Health	May-1997	May-2000
D09660	The role of endotoxin (lipid A) in host-pathogen interactions in *Salmonella* infections of mice	BBSRC	University of Cambridge	Sep-1998	Sep-2001
D09661	The role of endotoxin (lipid A) in host-pathogen interactions in *Salmonella* infections of mice	BBSRC	University College London	Nov-1998	Nov-2001
D09737	Role of IL12 in pathogenesis and immunity to *Salmonella*	BBSRC	Imperial College London	Oct-1998	Oct-2001
D10913	Control of BipA and its stress- and virulence-related targets in *Salmonella*	BBSRC	University of Southampton	Sep-1999	Sep-2002
D11217	Evaluation of cattle immune responses to live attenuated recombinant *Salmonella* vaccines	BBSRC	Institute for Animal Health	Sep-1999	Sep-2002
D11572	Role of secreted/outer proteins in immunity to *Salmonella*	BBSRC	University of Newcastle-Upon-Tyne	Oct-1999	Oct-2002
D11863	The relationship between iron metabolism and the growth, survival and pathogenicity of *Salmonella*	BBSRC	Institute for Animal Health, University of Reading	Dec-1999	Dec-2002
D13414	High-throughput whole-genome PCR with MADGE and SP-PCR for the analysis of genomic diversity in the enteric bacteria	BBSRC	University of Birmingham	Nov-2000	Feb-2004
D13422	High-throughput whole-genome PCR with MADGE and SP-PCR for the	BBSRC	University of	Nov-	Nov-2002

	analysis of genome diversity in the enteric bacteria		Southampton	2000	
D13683	Local events in the tissues of animals infected with *Salmonella*	BBSRC	University of Cambridge	Jan-2001	Jan-2004
D14755	Identification of immune subsets that contribute to IFN gamma-dependent protection against *S. typhimurium* in vivo	BBSRC	Institute for Animal Health	Nov-2001	Nov-2004
D15635	Characterisation of the effect of *Salmonella*-induced intestinal inflammation on pathogenesis	BBSRC	Institute for Animal Health	Oct-2001	Oct-2004
D16845	Toll-like receptors modulate the host immune response to infection with *Salmonella typhimurium*	BBSRC	University of Cambridge	Mar-2002	Mar-2005
D17313	Global identification of genes regulated by luxS- based quorum sensing in *Salmonella*	BBSRC	University of Newcastle-Upon-Tyne	Jan-2003	Jan-2006
D18179	A multidisciplinary study of *Salmonella*-protozoa interactions on surfaces, liquid culture and soil: bacterial phenotype dynamics	BBSRC	University of Warwick	Feb-2003	Feb-2006
D18830	Functional assessment of type III secreted Sop effector proteins from *Salmonella* in eukaryotic cells	BBSRC	Institute of Animal Health	Aug-2003	Aug-2006
D19269	Role of Salmonella pathogenicity island 4 in colonisation of cattle by *Salmonella* Typhimurium	BBSRC	Institute for Animal Health	May-2003	May-2006
D19920	Monitoring phagosomal metal levels during *Salmonella* infection	BBSRC	University of Manchester	June 2004	May 2007
D20030	Characterisation of *Salmonella* immunogens	BBSRC	University of Edinburgh	Apr-2004	Apr-2007
D20084	ShdA-mediated intestinal persistence of *Salmonella* Typhimurium	BBSRC	Imperial College	Dec-2004	Nov-2007
GAT09084	Development of methods for the identification and selection of mapped genes, targeting a recently mapped resistance gene in chickens	BBSRC	Institute for Animal Health, University of Liverpool	Nov-1998	Nov-2001
JE514316	The structural basis for infection: a nuclear magnetic resonance (NMR) approach	BBSRC	Imperial College, London	Mar-01	Mar-04
MMI09727/09738	Spatial dynamics and gene regulation of a bacterial-protozoa ecology	BBSRC	University of Bath, University of Edinburgh, University of	Jan-1999	Jan-2002

			Warwick		
P11074	The biochemistry and bioenergetics of bacterial thionate respiration	BBSRC	University of East Anglia	Jan-1999	Jan-2002
P18939	Flavohaemoglobin: a bifunctional NO-detoxifying enzyme for facultatively aerobic bacteria	BBSRC	University of Sheffield	Apr-2003	Apr-2006
PRS12194	A study of the starvation-stress response of *Salmonella enterica* using MudJ fusions and DNA microarrays	BBSRC	St Bartholomew's & the Royal London School of Medicine & Dentistry	Oct-1999	Oct-2002
PRS12222	Characterisation of the *Salmonella* Typhimurium extracytoplasmic stress response	BBSRC	University of Glasgow	Oct-1999	Oct-2002
S10274	Serotype specificity of *Salmonella*-host interaction *in vivo*	BBSRC	Institute for Animal Health	Oct-1998	Oct-2001
S14753	Characterisation of *Salmonella* net growth *in vivo* and serotype host-specificity	BBSRC	Institute for Animal Health	Aug-2001	Aug-2004
0177	Secreted virulence-associated proteins of *Salmonella*	BBSRC	Institute for Animal Health	Apr-1995	Mar-2001
0219	Role of secreted proteins (Sips) and their interactions with host cells in virulence studies of *Salmonella*	BBSRC	Institute for Animal Health	Jan-1997	Jan-2000
0237	Identification by positional mapping of genes influencing disease resistance	BBSRC	Institute for Animal Health	Apr-1997	Mar-2000
0238	Construction and characterisation of a resource population of pigs to map genes which influence resistance to salmonellosis	BBSRC	Institute for Animal Health	May-1998	Apr-2000
0239	Development of methods for the identification and selection of mapped genes, targeting a recently mapped resistance gene in chickens	BBSRC	Institute for Animal Health	Apr-1998	Apr-2001
0243	Resistance genes to *Salmonella*-carrier state in fowls	BBSRC	Institute for Animal Health	Jan-1999	Jun-2002
0244	Secreted effector proteins of *Salmonella*: structure, function and their role in virulence	BBSRC	Institute for Animal Health	Jun-1999	May-2002
0245	Mechanism of immunity to food-poisoning and vaccine serotypes of *Salmonella* in chickens	BBSRC	Institute for Animal Health	Jun-1999	May-2004
0246	Novel mechanisms of live, bacterial vaccines in protection against *Salmonella* and other food-borne zoonoses (A)	BBSRC	Institute for Animal Health	Apr-1999	Mar-2002
0302	Mechanisms behind host specificity of bacteria, investigated by use of host-	BBSRC	Institute for	May-	Apr-2001

	specific *Salmonella* serotypes		Animal Health	1997	
0396	Immunological responses induced by *Salmonella* in cattle	BBSRC	Institute for Animal Health	Apr-1997	Mar-1999
0455	Systems analysis for risk assessment	BBSRC	Silsoe Research Institute	Apr-2001	Mar-2004
0751	Serotype specificity of *Salmonella*-host interactions in vivo	BBSRC	Institute for Animal Health	Oct-1998	Oct-2001
0753	Genes of *Salmonella* Typhimurium involved in *in vitro* and *in vivo* stationary-phase growth	BBSRC	Institute for Animal Health	Oct-1998	Sep-2001
0755	Evaluation of cattle immune responses to live attenuated recombinant Salmonella vaccines	BBSRC	Institute for Animal Health	Sep-1999	Sep-2002
0756	Multi-component *Salmonella* live vaccines	BBSRC	Institute for Animal Health	Feb-2000	Jul-2003
0772	Evolutionary consequences of the control of *Salmonella* in chickens	BBSRC	Institute for Animal Health	Oct-1998	Sep-2001
0829	Microarray analysis of gene function and host interaction in *Salmonella* typhimurium (Salarray)	BBSRC	Institute for Animal Health	Dec-2000	Nov-2003
0856	Identification of immune subsets that contribute to IFNgamma- dependent protection against *S.* typhimurium *in vivo*	BBSRC	Institute for Animal Health	Nov-2001	Jun-2003
0858	Molecular basis of *Salmonella* virulence	BBSRC	Institute for Animal Health	Apr-2001	Mar-2004
0860	Characterisation of Salmonella net growth in vivo and serotype host-specificity	BBSRC	Institute for Animal Health	Jan-2001	Dec- 2003
0923	Characterisation of the effects of *Salmonella* induced intestinal inflammation on pathogenesis	BBSRC	Institute for Animal Health	Sep-2001	Aug-2004
1035	Functional assessment of type III secreted Sop effector proteins from *Salmonella* in eukaryotic cells	BBSRC	Institute for Animal Health	Jan-2003	Dec-2005
1037	Role of salmonella pathogenicity island-4 in colonisation of cattle by *Salmonella* Typhimurium	BBSRC	Institute for Animal Health	May-2003	Apr-2006
1116	Virulence-associated secreted and surface exposed bacterial proteins	BBSRC	Institute for Animal Health	Apr-2004	Mar-2007
41208	Molecular microbiology of *Salmonella* Typhimurium and *E. coli*	BBSRC	Institute of Food Research	Apr-2000	Apr-2005
41387	Gut immunology	BBSRC	Institute of Food Research	Apr-2001	Apr-2005

4321208	Molecular Microbiology of *Salmonella* Typhimurium and E. coli	BBSRC	Institute of Food Research	Apr-2000	Mar-2003
9912336	Toll-like receptors and the host response to *Salmonella enterica*	BBSRC	University of Cambridge	Jan-1999	Oct-2001
15003104	The significance of plant and microbial products in UK agriculture	BBSRC	Institute of Grassland and Environmental Research	Apr-1999	Mar-2002
9642	Improved procedures for the isolation and characterisation of salmonellas	DANI	DANI	Aug-1998	Aug-2000
9723	Microbial quality of beef carcasses in Northern Ireland abattoirs - A baseline study	DANI	DANI	Apr-1997	Mar-2000
9904	Epidemiological study of *Salmonella* infections in farm animals	DARD	DARD	Apr-2000	Apr-2004
OD2004	Loss of antibiotic resistance: analysis of phenotype and related gene expression	Defra	Veterinary Laboratories Agency	Apr-2000	Mar-2003
OD2005	A laboratory and field study to assess the potential for transfer of antibiotic resistance between bacterial strains in stored and spread organic wastes	Defra	Central Science Laboratory	Apr-2000	Mar-2003
OD2008	Transfer of antimicrobial resistance genes between bacteria in stored and spread farm wastes	Defra	Veterinary Laboratories Agency	Sep-2000	Aug-2003
OD2010	Use and abuse of non-antibiotic antimicrobials as major contributors toward the development of antimicrobial resistance	Defra	Veterinary Laboratories Agency	Oct-2003	Sep-2006
OZ0132	Antibiotic Resistance mechanisms in *Salmonella* and *Campylobacter*	Defra	Veterinary Laboratories Agency	Apr-1997	Mar-2000
OZ0134	Epidemiological studies of multiresistant *Salmonella* Typhimurium in Pigs	Defra	Veterinary Laboratories Agency	Apr-1997	Mar-2000
OZ0135	Epidemiological studies of multiresistant *Salmonella* Typhimurium in cattle	Defra	Veterinary Laboratories Agency	Apr-1997	Mar-2001
OZ0144	Constraints to uptake of adequate biosecurity on UK cattle and sheep farms, with special reference to zoonotic diseases.	Defra	Scottish Agricultural College,	Jun-2002	Nov-2003

			University of Reading		
OZ0311	Conventional vs capillary electrophoresis of RFLP fragments and PCR products for sensitive and rapid subtyping of *Salmonella*	Defra	Central Science Laboratory	Jun-1999	May-2002
OZ0312	Development of a sensitive and specific molecular typing method for the epidemiological study of *Salmonella*	Defra	Laboratory of the Government Chemist	May-1999	Aug-2001
OZ0313	Non Specific and Innate Resistance to *Salmonella* Infection in Chicken and Pigs	Defra	Institute for Animal Health	Apr-1999	Mar-2002
OZ0314	The role of defined bacterial genes and host genetic background in intestinal colonisation of poultry by *Salmonella*	Defra	Institute for Animal Health	Apr-1999	Mar-2002
OZ0315	*Salmonella* pathogenesis and immunity in cattle and pigs	Defra	Institute for Animal Health	Apr-1999	Mar-2002
OZ0316	Epidemiological studies of *Salmonella* in pigs and control by intervention	Defra	Veterinary Laboratories Agency	Aug-2000	Jun-2006
OZ0317	Epidemiological investigations of *Salmonella* contamination in table egg production	Defra	Veterinary Laboratories Agency	Aug-2000	Feb-2003
OZ0318	Understanding the dynamics of endemic and epidemic *Salmonella* infections in cattle and pigs: A comparative modelling approach	Defra	University of Liverpool	Oct-2002	Dec-2005
OZ0319	*Salmonella* pathogenesis in cattle and pigs	Defra	Institute for Animal Health	Jul-2002	Jun-2005
OZ0320	Bacterial and host genes in *Salmonella* colonisation in poultry	Defra	Institute for Animal Health	Jul-2002	Jun-2005
OZ0321	Investigation of the role of environmental contamination in the epidemiology of *Salmonella* infection in egg-laying flocks	Defra	University of Bristol, Veterinary Laboratories Agency	Oct-2002	Sep-2005
OZ0502	In vivo models to investigate the development of antibiotic resistance	Defra	Veterinary Laboratories Agency	Apr-2000	Mar-2002
OZ0711	Incidence and control of VTEC in animal feeds	Defra	ADAS	Aug-2002	Oct-2005

VF0101	University of Cambridge Veterinary Research Fellowship in Microbiology	Defra	University of Cambridge	Oct-1999	Sept-2004
VF0201	Liverpool Veterinary Research Fellowship in Epidemiology	Defra	University of Liverpool	Sep-1999	Aug-2004
VM02100	Factors influencing the development of resistance to fluoroquinolone antibiotics by food borne bacteria	Defra	Veterinary Laboratories Agency	Apr-2000	Sep-2003
VM02105	Identification and use of genomic markers of antibiotic resistance in campylobacters, salmonellae and enterococci.	Defra	Veterinary Laboratories Agency	Aug-2000	Jul-2003
VM02201	Modulation of dosing regimes to prevent development of fluoroquinolone resistance in bacteria (mainly *Salmonella* and *E. coli*) in chicken	Defra	Veterinary Laboratories Agency	Apr-2004	Mar-2006
VM0292	Protocol for field studies to monitor the persistence of resistance for up to 12 months after the withdrawal of antimicrobial feed activities	Defra	University of Glasgow	Oct-1999	Sep-2002
WA0656	Implications of potential measures to control pathogens associated with livestock manure management	Defra	ADAS	Nov-2000	Oct-2001
WA0804	Routes by which pathogens associated with livestock slurries and manures may be transferred from farm to the wider environment	Defra	ADAS	Sep-2001	Nov-2004
B01001	Physiological and microstructural factors controlling the survival and lag of food-borne pathogens	FSA	Institute of Food Research	Apr-1997	Aug-2000
B01006	Why do bugs stick to what they stick to?	FSA	Institute of Food Research	Apr-1998	Mar-2001
B01007	Development & study of tests to differentiate between tolerant & sensitive isolates of *Salmonella*	FSA	Health Protection Agency	Jul-1998	Jun-2001
B01008	Post stress detection of cold and hypochlorite stress proteins in *Salmonella* Typhimurium	FSA	University of Edinburgh	Oct-1998	Nov-2001
B01009	An assessment of population changes *Salmonella* Enteritidis and the emergence of strains with altered properties during food processing	FSA	Institute of Food Research	Apr-1998	Oct-2001
B01010/ B01011	Evaluation of the risk of induction and selection of more stress tolerant and virulent salmonellas by exposure to food production-related stress	FSA	Health Protection Agency	Jul-1998	Jun-2001
B01012	Dynamic database on microbial responses to common stresses in the food chain	FSA	Institute of Food Research	Apr-1999	Mar-2002
B01013	Genotypic subtyping of multiresistent *Salmonella* Typhimurium DT 104 from	FSA	Health	Jun-1999	Dec-2001

	food animals and humans		Protection Agency		
B01015	Determine exposure assessment & modelling risks associated with the preparation of poultry, catering & home	FSA	University of Wales - Cardiff	Jun-1999	Jun-2001
B01016	The evaluation and control of biofilm of significance to the food industry	FSA	University of Wales Institute, Cardiff	Dec-1997	Jul-1999
B01017	Review of the US FSIS *Salmonella* Enteritidis risk assessment model	FSA	HVR Consulting Services Ltd	Nov-1999	Aug-2000
B02005	Novel techniques for cleaning and decontaminating raw vegetables and fruit	FSA	Campden and Chorleywood Food Research Association	Apr-1998	Apr-2000
B02006	Cold Jet - A novel technique for cleaning and decontaminating food processing areas, equipment and foodstuffs.	FSA	Microchem Biosciences Ltd	Feb-1999	Dec-2001
B02010	The evaluation and application of information on consumer hazard and risk to food safety education	FSA	University of Wales Cardiff	Jul-1999	Jun-2001
B02013	A study to examine the contamination of catering and economy sausages with *Salmonella* spp.	FSA	Health Protection Agency	Mar-2000	Oct-2000
B02015	A national survey of potential cross-contamination resulting from kitchen cloths in domestic kitchen	FSA	Central Science Laboratory	Aug-2000	Mar-2002
B02016	Microbiological risk factors associated with the domestic handling of meat	FSA	Campden and Chorleywood Food Research Association	Nov-2000	Oct-2002
B03001	Field studies to identify and evaluate key intervention points for *Salmonella* control during Broiler production	FSA	Veterinary Laboratories Agency	Jul-1997	Jun-2000
B03002	Risk factors of cross infection by *Salmonella* spp from fresh poultry packaging in retail stores	FSA	Campden and Chorleywood Food Research Association	Sep-1998	Nov-1999
B03005/6/7	A Review of measures to reduce levels of *Salmonella* and *Campylobacter* in	FSA	ADAS, Silsoe	Sep-	Aug-1999

	poultry and development of an appropriate risk assessment model		Research Institute, University of Nottingham	1998	
B03008	Studies to identify critical points for infection of live birds or contamination of poultry carcasses with *Campylobacter* and *Salmonella*	FSA	Health Protection Agency	Apr-1998	Nov-02
B03010	Efficacy of water disinfection systems for broiler production unit	FSA	University of Aberdeen	Oct-1999	Jun-2002
B03012	Environmental survival of *Salmonella* serotypes Typhimurium DT104 and Enteritidis PT4: The role of surface antigens	FSA	Health Protection Agency	Aug-1998	Oct-2000
B03015	A study to examine the egg-to-egg variations in the growth of *Salmonella* spp. In egg contents	FSA	Health Protection Agency	Oct 2000	Mar-2002
B03016	Cross contamination from the external surface of eggs in relation to risk of exposure to *Salmonella*	FSA	London Metropolitan University	Sep-2000	Sep-2003
B03017	A review of commercial egg washing with particular emphasis on the control of salmonella	FSA	ADAS	Oct-2000	Sep-2002
B05003	Pathogens in organic wastes: their levels and survival both during storage and following application to agricultural land	FSA	ADAS	Jul-1999	Dec-02
B05008	The levels of pathogens in abattoir wastes	FSA	University of Bristol	Nov-1999	Feb-2002
B08008	Methods used for the assessment of the number and prevalence of *Salmonella* and *Campylobacter* spp. in chicken on retail sale	FSA	Health Protection Agency	Nov-1999	Jun-2000
B09005	Review of microbiological methods in the food industry	FSA	Campden and Chorleywood Food Research Association	Jun-1998	May- 2000
B09008	Accelerated detection of *Salmonella* and verocytotoxin-producing *E. coli* in food	FSA	University of Reading	Jun-1999	Jun-2002
B10001	Molecular epidemiology of multiple drug resistance and resistance to fluroquinolone antibiotics in *S. Typhimurium* DT104 and related phage types from humans and food animals	FSA	Health Protection Agency	Jan-1999	Sep-2001
B12004	Evaluation of the growth and survival of *Salmonella* on chilled poultry under	FSA	Campden and	Apr-2003	Jun-2003

	different storage temperatures.		Chorleywood Food Research Association		
B14004	Generation of an archive of extracted nucleic acid for the IID archived faecal specimens	FSA	Health Protection Agency	Jan-2003	Dec-2007
B15003	To make recommendations on the best practical procedures to sample and test poultry flocks for *Salmonella*	FSA	Direct Laboratory Services	Nov-2003	Aug-2004
B17002	Assessment of the risks to food safety associated with spreading of animal manure and abattoir waste on agricultural land	FSA	Water Research Centre	May-2001	Apr-2002
B18002	UK-wide survey of *Salmonella* and *Campylobacter* in fresh and frozen chicken on retail sale	FSA	ADAS	Mar-2001	Jun-2001
B18007	UK-wide survey of *Salmonella* contamination of eggs	FSA	Direct Laboratory Services	Mar-03	Jun-03
M01002	Establishment of critical control points for enteric pathogens in pork production	FSA	University of Nottingham	Mar-1999	Mar-2001
M01009	Source and spread of particulate and bacterial contamination between cattle during the farm-to-abattoir phase of the production cycle	FSA	ADAS	Oct-1999	Sep-2003
M01011	A Study of Factors affecting MHS scores of sheep arriving at abattoirs and bacterial contamination of their carcasses	FSA	ADAS	Jan-2000	Dec-2000
M01013	Farm management practices to improve the visible and microbiological cleanliness of cattle hides at slaughter	FSA	ADAS	Jan-2000	Sep-2003
M01014	Microbiological verification of HACCP in meat plants	FSA	Royal Veterinary College	Apr-2000	Apr-2001
M01015	Factors affecting the presence and spread of human bacterial pathogens in sheep	FSA	ADAS	Jan-2000	Dec-2003
M01017	Standardisation of sampling and analysis in poultry abattoirs in support of HACCP-based hygiene solutions.	FSA	Direct Laboratory Services	Oct-2001	Sep-2004
M01019	Physical methods readily adapted to existing commercial lines for reducing pathogens, particularly campylobacters, on raw poultry	FSA	University of Bristol	Oct-2001	Mar-2004
ZB00023	Review of testing and scheduling practices for *Salmonella* and *Campylobacter*	FSA	ADAS	Jan-2002	Jul-2005

	undertaken by the UK poultry industry and to determine the factors affecting their use				
ZB00033	Poultry transport crate hygiene	FSA	University of Bristol	Jan-2002	Jul-2005
ZB00034	Biosecurity on the broiler farm as an anti-*Salmonella* control agent	FSA	University of Bristol	Jan-2002	Jul-2005
00-RESR-046	Detection and molecular characterisation of selected pathogenic organisms isolated in unpasteurised milk using milk filters. Follow-on surveillance and risk assessment for MAP culture positive and suspect positive herds	FSPB - Food Safety Promotion Board	Queens University Belfast	Jan-2001	Dec-2004
03-RESR-005	Development of a risk assessment model for *Salmonella* in shell eggs and processed eggs in Ireland	Food Safety Promotion Board	Food Safety Promotion Board	May-2004	May-2007
FS1319	Development of a novel bacterial trap to capture and concentrate low numbers of pathogens from large volumes of food homogenate	MAFF - Food Hygiene Division	Central Science Laboratory	Apr-1997	Mar-1999
FS1532	Characterisations of bacterial membrane damage associated with electric field and high pressure treatment	MAFF - Food Hygiene Division	University of Surrey	Sep-1996	Aug-1999
FS3206	Efficacy testing of disinfectants used in the food industry against a range of pathogens including *E. coli* O157	MAFF - Food Hygiene Division	Institute for Animal Health	May-1998	Nov-1999
UMA/001/95	Rapid luminescent detection and enumeration of low numbers of viable specific micro-organisms	Scottish Office Agriculture Environment and Fisheries Department	University of Manchester	Jan-1996	Jun-1999
RRI/504/95	Antagonistic interactions between gut micro-organisms	SEERAD	Rowett Research Institute	Apr-1995	Mar-2000
SAC/136/97	Investigation of the mechanisms for the adherence and penetration of the gut by food-borne bacterial pathogens	SEERAD	Scottish Agricultural College	Apr-1997	Sep-2000