UK Publicly Funded Research Relating to *Salmonella*: Update, May 2009

Research covered from January 2004 to May 2009

Report of the Microbiological Safety of Food Funders Group

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UK PUBLICLY FUNDED RESEARCH RELATING TO *SALMONELLA*: UPDATE ON RESEARCH FROM 2004 TO MARCH 2009

SUMMARY

This report provides an update to the Microbiological Safety of Food Funders Group (MSFFG) publication *UK Publicly Funded Research Relating to Salmonella*[1], with the addition of information from new research projects funded between 2004 and May 2009 by the MSFFG member organisations.

In the UK food-borne *Salmonella* infections are most commonly caused by *Salmonella* Enteritidis or *Salmonella* Typhimurium, that give rise to symptoms of diarrhoea, stomach cramps, vomiting and fever. Acute symptoms may last for 1 to 2 days or may be prolonged, depending on host factors, ingested dose, and strain characteristics. The reported numbers of infections with *S.* Enteritidis in the UK rose during the 1980s and 1990s, and then declined from 1998 onwards. Infection levels with *S.* Typhimurium, have been maintained at a comparatively lower level than that of *S.* Enteritidis from the mid-1980s, and there has been a gradual decline in reported cases.

Salmonellosis has mainly been associated with the consumption of undercooked contaminated meat especially poultry, pork and beef, and from undercooked eggs and eggs containing dishes. Although less common, salmonellosis has also been associated with consumption of contaminated ready to eat fruit and vegetables. Cross-contamination can also occur to food preparation surfaces, kitchenware, towels and other foods. Outbreaks tend to occur from the consumption of contaminated commercially manufactured or prepared food and also from infections acquired outside the UK by returning travellers. The reported incidence data of food-borne *Salmonella* infection in the UK in 2008 was 18.8 cases per 100,000 of the population. In comparison to other food-borne zoonoses, the incidence rate for *Salmonella* in 2008 was second to *Campylobacter* with an incidence rate of 90.9 cases per 100,000. Verocytotoxin producing *Escherichia coli* O157 with a rate of 1.9 cases per 100,000[2] was the third most commonly reported food-borne bacterial illness in the UK in 2008.

Projects summarised here include those in which the genetic and physiological basis of *Salmonella* pathogenicity in the host animal, and the subsequent host response to infection has been investigated. The association of salmonellae with food continues to be examined with studies on poultry and meat production, and the egg industry. Epidemiological projects have also been crucial to understanding the relationship between food and outbreaks and consumer practices. The global concerns about the

[1] http://www.food.gov.uk/multimedia/pdfs/version_.PDF

[2] The Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents and Food-Borne Outbreaks in the European Union in 2008, *The EFSA Journal* (2010)

increase in antimicrobial resistance in *Salmonella* have been met with research aimed at identification of antimicrobial resistant genes. Vaccination strategies targeting a reduction in *Salmonella* in animals have met with success and projects are ongoing to try to improve on current vaccinations and to optimise the immune responses in animals.

Significant advances achieved through research

The availability of the genome sequences for a number of *Salmonella* serovars, and the associated use of a wide range of molecular biology tools, have been central to the advances in our understanding of *Salmonella* infection. Examples of the utility of molecular methods include the identification and regulation of virulence factors, the responses of bacteria to the host environment, identification of antimicrobial resistance genes and the use of Polymerase Chain Reaction (PCR) based assays for screening. As the use of molecular methods becomes more widespread they have become incorporated in large scale surveillance studies and have provided more sensitive and specific tests for the identification of salmonellae.

Work on vaccines and antimicrobial resistance has also benefited enormously from genetic technology, generating increased insights into antimicrobial resistance genes and host immune responses.

Surveillance studies assessing the incidence of *Salmonella* serovars in the poultry, meat and egg industries have been influenced by European legislation; EU Zoonoses directive 2003/99/EC concerns the monitoring of zoonoses and zoonotic agents and regulation (EC) 2160/2003 concerns the control of *Salmonella* and other specified food-borne zoonotic agents. The UK implementation of these directives by regular monitoring of layer and broiler flocks and slaughter animals, and vaccinations in flocks has contributed to a continued decline in the reported incidence on *Salmonella* cases and also a reduction in *Salmonella* levels in poultry and eggs produced in the UK.

Outstanding issues

The cumulative effect of the genomic research in *Salmonella* has led to a significant increase in the overall understanding of *Salmonella* infection, antimicrobial resistance and vaccination strategies. There remains a need to ensure that this knowledge is translated into the issues associated with *Salmonella* food-poisoning in humans.

While the decline in reported *Salmonella* cases is encouraging, outbreaks of salmonellosis are still occurring in the UK. There is therefore a need to continue animal, food and human surveillance, epidemiology studies and studies on consumer practices and to identify patterns in the infection routes.

LAY SUMMARY

This report summarises research projects in the UK funded by the MSFFG between 2004 and 2009 on *Salmonella* and highlights potential gaps in the research programmes.

Salmonella enterica is the name of one of the main species of bacteria which can cause food poisoning, the symptoms of which includes diarrhoea, stomach cramps, vomiting and fever. Most people recover but some can die from complications that occur following infection e.g. septicaemia, which can occur if bacteria enter into the bloodstream. Fatalities are more common in the very young or elderly or in people with some other underlying illness.

Salmonella bacteria are commonly found in the digestive system of farm animals. Meat can become contaminated from contact with faeces during its production at abattoirs. *Salmonella* are also found in egg contents as well as on the shells. Although less common, food poisoning from *Salmonella* has also been associated with consumption of contaminated ready to eat fruit and vegetables. Cross-contamination can also occur where *Salmonella* is transferred onto table-tops, kitchenware, towels or to other foods; items which themselves can be the source of further transfer. *Salmonella* infection occurs when a sufficient dose of the bacteria enters the human digestive system. This can be by the consumption of undercooked contaminated meat, especially chicken, pork and beef, from undercooked eggs or egg containing dishes, or from the consumption of contaminated ready-made meals and/or pre-cooked cold meats which have not been prepared hygienically. Contamination tends to occur following the use of poor hygiene standards either in food preparation settings or in the home; if good standards are followed then food which is produced is unlikely to cause food poisoning.

Research has been carried out in the UK to better understand how salmonellae cause food-poisoning and how to prevent infection. A range of topics are investigated which include; how *Salmonella* are able to cause an infection, whether there are ways of reducing their levels in meat and eggs by vaccinating farm animal and how *Salmonella* are developing resistance to antimicrobial agents. Molecular biology techniques that look at which genes are present in the bacteria and how they influence the behaviour of the bacteria are often used to help researchers. Other projects continue to test farm animals to see if they harbour *Salmonella,* and others assess the causes behind outbreaks amongst groups of people.

Research is key to understanding how *Salmonella* infects humans and animals and how it is causes food-poisoning. Knowledge in such fields may help to reduce the risk of being infected with *Salmonella*. As well as the research carried out in the UK, there are many research projects in Europe, America and across the world. In the UK there has been a decrease in the number of cases of *Salmonella* in humans since 1998. This has occurred

partly by the introduction of monitoring and vaccinations of farm animals which have been undertaken to comply with new regulations in Europe which aim to reduce the numbers of *Salmonella* infections.

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INTRODUCTION

1. BACKGROUND

1.1 The Microbiological Safety of Food Funders Group (MSFFG) has previously published two reports summarising *Salmonella* research projects in relation to the microbiological safety of food which are funded by the MSFFG member organisations. The first report *UK Publicly Funded Research Relating to Salmonella*[3] covered research projects funded between 1990 and the end of March 2000. This report was updated in 2005[4] to include research projects carried out between 1999 and 2004. The updated report is described as the "2005 *Salmonella* MSFFG report" within subsequent sections of this document. Since 2004 a number of new projects on *Salmonella* have been commissioned and been recorded in the MSFFG database[5], and therefore it is timely to re-examine the research and highlight recent progress in the science of the pathogenicity of this organism and its importance as a hazard in the food chain.

1.2 In 2003 the EU Regulation (EC) 2160/2003[6] *on the control of Salmonella and other specified food-borne zoonotic agents,* was published and has led to the introduction of targets for reducing *Salmonella* levels in animals used in primary production. In support of measures put in place to assist in meeting these targets, a number of National Control Programmes (NCP's) have been initiated (see Sections 1.14 – 1.17). (see www.defra.gov.uk/animalh/diseases/zoonoses/ncp.htm).

1.3 *Salmonella* are Gram-negative, rod-shaped bacteria which live in the intestinal tracts of animals. Some serovars are ubiquitous, while others are specifically adapted to a particular host. Many species of animal including amphibians, birds, domesticated and wild mammals, insects and reptiles can carry *Salmonella.*

1.4 The nomenclature for *Salmonella* has changed in recent years as methods have become available to examine the serovars. *Salmonella* nomenclature was originally based upon names allocated according to the clinical role of *Salmonella,* with strains considered different species (and therefore italicised) when isolated from the clinical condition, e.g. *Salmonella typhi, Salmonella cholerae-suis, Salmonella abortus-ovis.* Since the host specificity suggested by some of these earlier names does not exist (e.g. *S. typhimurium and S. cholerae-suis* are ubiquitous), names derived from the geographical origin of the first isolated strain of the newly discovered serovars were next chosen, e.g., *S. london, S. panama, S. stanleyville.* Subsequently, with the adoption of serological

[3] http://www.food.gov.uk/science/research/researchinfo/foodborneillness/microfunders/msffg/55669
[4] http://www.msffg.org.uk/reports/reports/*Salmonella*2.pdf
[5] http://www.msffg.org.uk/
[6] http://eur-lex.europa.eu/LexUriServ/site/en/oj/2003/l_325/l_32520031212en00010015.pdf

analysis, Kauffmann (1961) defined the species as a "group of related serofermentative phage types" with each *Salmonella* serovar considered as a species.

1.5 More recently it was found that all *Salmonella* serovars form two distinct DNA hybridization groups, i.e. that the *Salmonella* genus consists of two species. These are defined as *Salmonella enterica* (species 1), and *Salmonella bongori* (species 2).

Of these two species *Salmonella enterica,* is divided into subspecies, as follows:

Subspecies I - *S. enterica* subspecies (subsp.) *enterica*
Subspecies II - *S. enterica* subsp. *salamae,*
Subspecies III - *S.* Arizona [IIIa *S. enterica* subsp. *arizonae,*; IIIb *S. enterica* subsp. *diarizonae*];
Subspecies IV - *S. enterica* subsp. *houtenae*
Subspecies VI - *S. enterica* subsp. *indica*

Species 2 – *Salmonella bongoris* – was formerly called subspecies V *S*. enterica subsp. bongori, but this designation has now been superceded.

Each subspecies contains various serovars defined by a characteristic antigenic formula.

1.6 There have been over 2,500 serovars of *Salmonella*[7] characterised. Many serovars of *Salmonella,* which had been previously described as species fall within the species *Salmonella enterica* subsp. *enterica* including those originally referred to as *Salmonella typhimurium*, *Salmonella enteritidis, Salmonella paratyphi,* and *Salmonella typhi.* These are now defined as, for example, *Salmonella enterica* subsp. *enterica* serovar Typhi, which may be shortened to *S.* Typhi (non italicised). This is now a commonly accepted practice and is adopted in the following sections of this report.

1.7 In humans *S*. Typhi infection can cause typhoid fever, resulting from bacterial invasion of the bloodstream, but most food-borne *Salmonella* serovars cause acute gastroenteritis. Food-borne *Salmonella* infections are caused by ubiquitous *Salmonella* serovars (most commonly *S*. Enteritidis and *S*. Typhimurium). Symptoms appear (diarrhoea, stomach cramps, vomiting, and fever) between 6-48 hours following ingestion of contaminated food and acute symptoms may last for 1 to 2 days or may be prolonged, depending on host factors, ingested dose, and strain characteristics. Recovery usually occurs without the need for medication, but complications can arise which include sepsis, meningitis and reactive arthritis. Chronic and extra-intestinal infections require antimicrobial therapy.

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

1.8 *S.* Typhi and *S.* Paratyphi A cause typhoid (enteric fever) or typhoid-like fever and are human serovars. *Salmonella* organisms in these cases are transmitted through human faecal contamination of water or food. Infection tends to be limited to those countries with poor control of water or sewage systems, although travellers returning from such countries may bring these infections into the UK.

1.9 Figure 1 shows the number of reported isolates of *Salmonella* per 100,000 of the population in England and Wales, Scotland and Northern Ireland between 1990 and 2008. The levels in the early 1990's, were followed by a decline in numbers from 1998 onwards in all UK countries, a trend which has continued to be maintained up to 2008. In 2007 the origins of the cases were distributed between those occurring in the UK (24%) those which originated from a non-UK source (21.4%) and those with an unknown source (54.6%)[8]. Under ascertainment of Infectious Intestinal Disease (IID) is well recognised, and the true population burden is greater than that suggested by surveillance. For every report to national surveillance for disease caused by *Salmonella* spp. there are estimated to be approximately three unreported cases in the community[9].

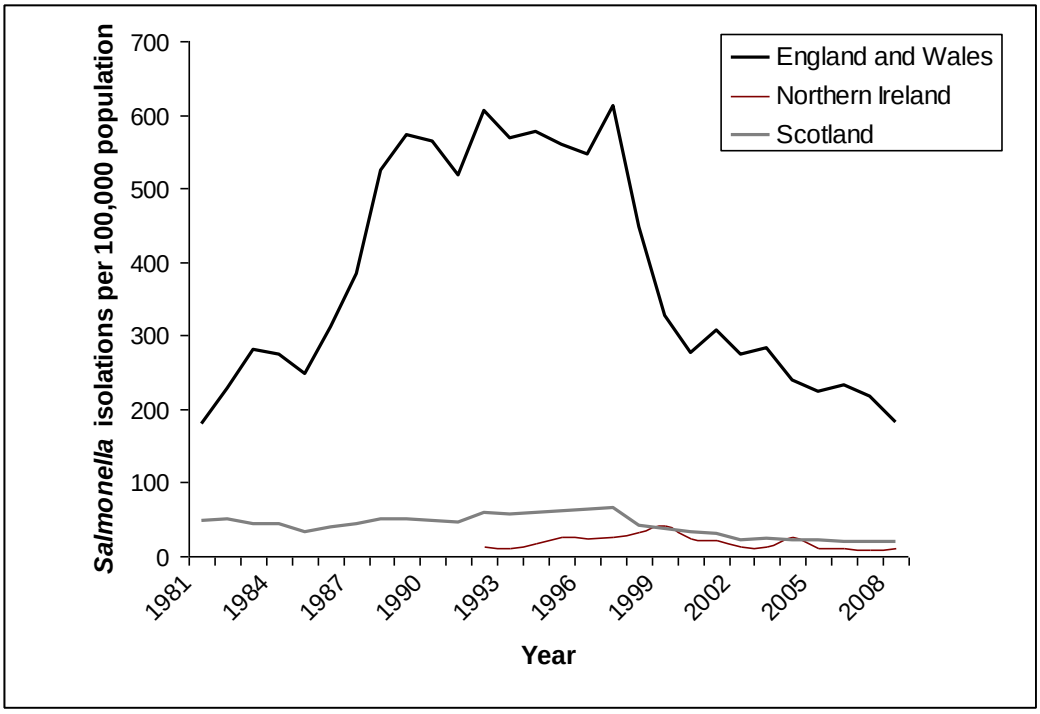

Figure 1: Number of laboratory report of isolates of *Salmonella* (excluding *S.* Typhi and *S.* Paratyphi) per 100,000 of the population in the UK.

[8] The community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union in 2007, *The EPSA Journal* (2009), 223. *www.efsa.europa.eu/en/scdocs/scdoc/223r.htm*

[9] Report of the Study of Infectious Intestinal Disease in England*. Infectious Intestinal Disease Study Executive Committee,* 2000. Research project funded by the Department of Health. Published by the Food Standards Agency.

1.10 The most common serovar associated with food-borne salmonellosis in the UK is *S.* Enteritidis and this can be demonstrated in the annual totals in England and Wales shown in Figure 2. The number of reported *Salmonella* infections in the UK during the 1990s, and the ongoing decline from 1998 onwards (Figures 1 and 2) has been due mainly to the decline in number of *S.* Enteritidis infections. Infection levels with serovar *S.* Typhimurium, have also shown a gradual decline, and the other *Salmonella* serovars have remained at a stable level since the mid 1980s.

1.11 Salmonella serovars of epidemiological importance such as Enteritidis and Typimurium are subdivided by phage typing, and within *S.* Enteritidis, over 80 *phage types* (PT) have been identified. In the early 1990's PT4 was the most commonly reported phage type of *S.* Enteritidis. More recently numbers have declined and are now comparable to those seen for the other phage types (Figure 3) which have remained relatively constant since 1981.

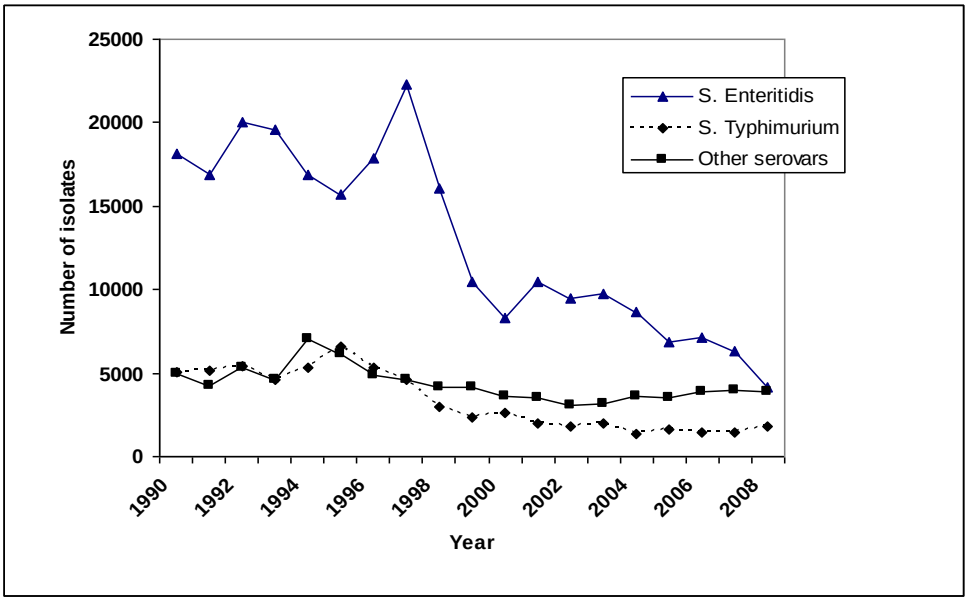

Figure 2: Number of laboratory reports of Salmonella serovars in England and Wales 1990 – 2008. Reports are of lower gastrointestinal isolates of *Salmonella* in humans (excluding *S*. Typhi; *S*. Paratyphi). Data reported to the Health Protection Agency Centre for Infections.

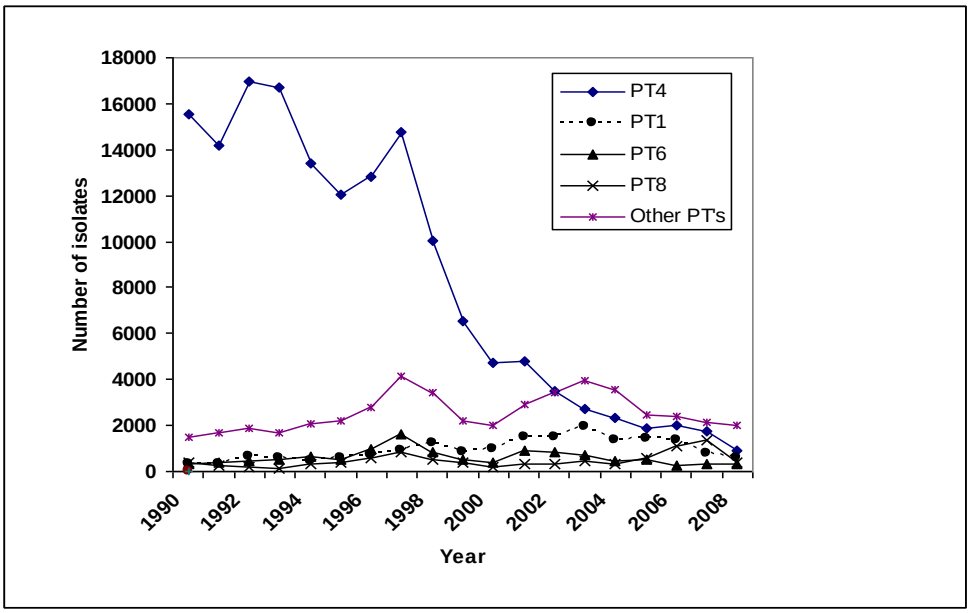

Figure 3: Number of laboratory reports of *S.* Enteritidis phage types from humans in England and Wales 1990 – 2008. Reports are of lower gastrointestinal isolates reported to the Health Protection Agency Centre for Infections.

1.12 Farm animals, especially - poultry, pigs and cattle, represent an important reservoir of *Salmonella*. Many animals are carriers of *Salmonella*, and show no sign of infection, and these organisms can be transmitted to humans through consumption of contaminated foods. Contamination of meat may originate from animal salmonellosis, from contamination by the intestinal contents during evisceration of animals, or from the hide/fleece during removal and subsequent washing, or transportation of the carcasses. Microbiological contamination on the surface of the meat is usually of little concern providing the meat is properly cooked, although handling of contaminated meat may result in a potential risk of cross-contamination of hands, tables, kitchenware, towels, and other foods. However, if contaminated meat is minced, *Salmonella* may become incorporated within the meat product which if inadequately cooked, may lead to ingestion of *Salmonella* and a subsequent infection.

1.13 A variety of salad vegetables, spices, and herbs have also been implicated in outbreaks of infection[10]. Likewise, infection can also occur following ingestion of dairy food that could harbour *Salmonella* such as cream or mayonnaise, or infant formula.

1.14 Salmonellae can also be found associated with eggs and can be present on the egg shells from faecal contamination from the hen, and *S*. Enteriditis in particular can contaminate the egg contents from infected ovaries before the shells are formed.

[10] Zoonoses Report United Kingdom (2007) Department for Environment, Food and Rural Affairs.http://www.defra.gov.uk/foodfarm/farmanimal/diseases/atoz/zoonoses/documents/reports/zoonoses2007.pdf

1.15 EU Regulation (EC) 2160/2003[11] *on the control of Salmonella and other specified food-borne zoonotic agents* was published in 2003 and has led to the introduction of targets for reduction of *Salmonella* levels in primary production. As part of the measures put in place to assist in meeting these targets, National Control Programmes (NCP's) have been initiated (see http://www.defra.gov.uk/animalh/diseases/zoonoses/ncp.htm). The aim of the NCPs is to protect human health by auditing flocks against agreed targets to reduce the prevalence of certain zoonoses in animal populations at primary production level, and where necessary, other stages of the food chain. Audits cover farm animal species which present a potential risk of transmitting *Salmonella* and other zoonotic agents to humans. These are currently restricted to poultry (breeding and laying flocks of hens, broilers and turkeys) and pigs (herds of slaughter and breeding pigs).

1.16 Surveys and investigations of eggs for the presence of *Salmonella* have played an important role in the UK in understanding the extent and pattern of contamination. An EU survey carried out during 2004-2005 identified 7.9% of laying hen holdings to be positive for *S*. Enteritidis or *S.* Typhimurium in the UK[12]. A 2003 survey of UK-produced eggs indicated a level of 0.34% positive samples (boxes of six eggs) for *Salmonella*[13]. UK demand is greater than UK supply and eggs are sourced from other EU countries where vaccination of layer flocks may be more variable. In a 2003-2006 survey in the UK of non-UK eggs 9% of boxes of six eggs had shell contaminated with *Salmonella* and 6.4% of the 9% were also contents positive[14]. An FSA survey in 2007 detected *Salmonella* spp. in 0.38% of pooled samples of egg shell[15].

1.17 More recently across both egg production and poultry production breeding lines the estimated prevalence based upon detection of *Salmonella* in flocks under the requirements of the *Salmonella* National Control Programme in breeding chickens in the UK in 2007 was reported to be 1.9% for all types of breeding flocks for the five salmonellae of public health significance, (*S*. Enteritidis, *S*. Typhimurium, *S.* Hadar, *S*. Virchow and *S*. Infantis)[16].

1.18 Between 2006 and 2007 studies on the prevalence of *Salmonella* were carried out in turkey flocks and in pigs at slaughter to establish baseline prevalence of *Salmonella* under Directive 2003/99/EC. In UK turkey flocks the level of *Salmonella* was estimated at 33.2% (EU level 3.7%) of

[11] http://eur-lex.europa.eu/LexUriServ/site/en/oj/2003/l_325/l_32520031212en00010015.pdf
[12]http://www.efsa.europa.eu/cs/BlobServer/Report/zoon_report_ej97_finlayinghens_en,0.pdf?ssbinary=true
[13] http://www.food.gov.uk/multimedia/pdfs/fsis5004report.pdf
[14] http://www.eurosurveillance.org/viewArticle.aspx?ArticleId=3086
[15] http://www.food.gov.uk/multimedia/pdfs/eggsurvey2007.pdf
[16] The community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union in 2007, *The EPSA Journal* (2009), 223. *www.efsa.europa.eu/en/scdocs/scdoc/223r.htm*

flocks that were reared for human consumption and 4.4% in breeding turkey flocks (EU 13.6%). *S.* Enteriditis and *S.* Typhimurium (ST) were detected in 4.6% of UK flocks (ST only) in comparison to 3.8% for the EU and 0.5% of UK breeding flocks (EU levels 1.7%). The baseline incidence for pigs in the UK was obtained by sampling at slaughter houses. *Salmonella* was found to be present in 21.2% of lymph nodes (EU weighted prevalence 10.3%)[17]. A 2007-2008 survey of chicken at retail by the FSA found the level of *Salmonella* prevalence to be 6.6%[18].

2. RESEARCH FUNDED BY OTHER FUNDING BODIES

Within the UK

2.1 In the UK research into *Salmonella* is also supported by funding bodies other than the BBSRC, FSA and Defra. A number of projects on salmonellae are funded by the MRC and Wellcome Trust, but may have been excluded from inclusion here as they do not specifically include research into *Salmonella* in relation to food- borne illnesses. Similarly, projects using other model organisms or systems and which may have broader relevance have not been considered.

2.2 A search of projects funded by the Wellcome Trust between September 2003 and September 2008 identified eleven project titles containing the word "*Salmonella*". The main focus of the research was the pathogenicity of *Salmonella* and the host immune response (5/11). Other project topics included investigations of bacterial physiology and genetics and epidemiology studies. The Wellcome Trust also funded the project *'International Partnership Research Award in Veterinary Epidemiology (IPRAVE)* [19] between 1999 and 2005 during which over 7500 isolates of food-borne zoonotic bacteria including *S.* Typhimurium were collected and made available to the scientific community.

2.3 The Wellcome trust is also the primary funder of the Sanger Institute which carries out large scale genome sequencing of many organisms including bacterial pathogens. Eleven *Salmonella* serovars have been investigated and by June 2009 five sequences were completed, which include two *S*. Typhimurium strains, *S*. Hadar and *S*. Bongori. One serovar sequence is near completion, and five are ongoing including *S*. Enteritidis PT4 and *S*. Typhi[20]. The availability of the genome sequence of *Salmonella* serovars is of key importance to the continued effort in understanding the pathogenicity of these bacteria and is reflected

[17] The community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union in 2007, *The EPSA Journal* (2009), 223. *www.efsa.europa.eu/en/scdocs/scdoc/223r.htm*

[18] http://www.food.gov.uk/science/surveillance/fsisbranch2009/fsis0409

[19] http://www.wellcome.ac.uk/Professional-resources/Biomedical-resources/Micro-organisms/WTD020697.htm

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

in the number of MSFFG projects that include a genomic aspect to the investigation.

2.4 A search for "*Salmonella*" in the Medical Research Council (MRC) research database[21] identified records of six grants awarded between 2004 and May 2009, four of which address host responses to *Salmonella* infection with the remaining two projects focussing on antibiotic resistance.

Within EU funded Europe

2.5 The Community Research and Development Information Service (CORDIS)[22] holds a R&D database containing details of projects from 1986 onwards that have been wholly or partially financed from the budget of the European Communities. A database search from 2004 onwards using the term "*Salmonella*" identified a total of 22 projects of which 16 were relevant to *Salmonella* as a food-borne disease. Research fields ranged from basic research on *Salmonella* pathogenesis, to those projects investigating prevention of *Salmonella* in meat and egg production and also within animal husbandry. Projects concerning standards and policies, new diagnostic tests and computer models of infection and alternatives to antibiotics were also identified in this cohort. Six of the 16 projects listed the UK as the lead participant, with Spain leading three projects, Sweden leading two projects and France, Netherlands, Austria, Belgium and Portugal each the lead participant for one project.

2.6 The European Centre for Disease Prevention and Control (ECDC) manages a programme on food- and water-borne diseases and zoonoses (FWD). This programme was set up in 2006, covers 20 diseases and has the general objectives to improve surveillance in the EU, and increase knowledge of aetiology, prevention and control, early detection and co-ordinated response to outbreaks. Project activities are largely based on achievements of different surveillance networks that are, or have been, functioning at the EU level. The coordination of the former network Enter-net was transferred to ECDC in 2007. In 2008, a new surveillance network was set up to focus on six priority diseases: salmonellosis, campylobacteriosis, VTEC/STEC infection, listeriosis, shigellosis and yersiniosis. The first annual meeting with the new FWD network members was held in October 2008.

2.7 The European Cooperation in Science and Technology (COST) funded a European Communities project: ACTION 920 *Foodborne Zoonoses: a Coordinated food chain approach* which was completed in 2006. The main objective of this project was to develop and improve the coordination and communication between experts in the different food

[21] http://www.mrc.ac.uk/Utilities/Documentrecord/index.htm?d=MRC002629
[22] http://cordis.europa.eu

chain sectors in order to improve the coordination and control of food-borne pathogens.

2.8 In 2004 the Sixth Framework Programme (FP6A) 'Food quality and safety' theme funded the EU MED-VET-NET network which aimed to unite medical and veterinary expertise in the fight against zoonoses and make better use of resources by sharing facilities internationally, centralising archives of reference material and standardising procedures to enable information to be pooled. A number of EU MED-VET-NET projects relevant to *Salmonella* can be found in the MSFFG database and are listed in Appendix 1.

Within the US

2.9 The office of Extramural Research at the National Institutes of Health maintains the CRISP (Computer Retrieval of Information on Scientific Projects) database[×]. This database includes projects funded by the National Institutes of Health (NIH), Substance Abuse and Mental Health Services (SAMHSA), Health Resources and Services Administration (HRSA), Food and Drug Administration (FDA), Centers for Disease Control and Prevention (CDC), Agency for Health Care Research and Quality (AHRQ), and Office of Assistant Secretary of Health (OASH).

2.10 The database contained 966 recorded entries on *Salmonella* between January 2004 and May 2009, many of which are multiple entries of the same project. Summarising this list, there were 132 projects with either *Salmonella* in the title or some relevance to *Salmonella* as a food-borne disease. Many of the topics under investigation are in line with those captured in the MSFFG database. These include a significant amount of research on pathogenicity and virulence of *Salmonella*, and also the physiological and immunological response of the host. Projects describing work upon vaccination strategies and antimicrobial resistance were also identified.

Global initiatives

2.11 The department of Food Safety, Zoonoses and Food-borne Diseases (FOS) at the World Health Organisation (WHO)[23] is developing a comprehensive strategy on strengthening surveillance of food-borne disease. In September 2006 it convened an international consultation during which FOS and its multiple partners assessed the currently available evidence, and charted the strategic way forward to fill surveillance data gaps. This meeting prompted the launch of an initiative to estimate the global burden of food-borne diseases from all major causes through the food-borne disease burden epidemiology reference group (FERG)[24] which comprises over 30 internationally renowned

[×] The CRISP database has recently been superseded by the National Institute of Health's RePORT Expenditures and Results (RePORTER) query tool. http://report.nih.gov

[23] http://www.who.int/foodsafety/en/

[24] http://www.who.int/foodsafety/publications/foodborne_disease/burden_sept06/en/index.html

experts in a broad range of disciplines relevant to global food-borne disease epidemiology.

2.12 In 2000 the WHO also created the Global Salm-Surv (WHO GSS)[25] network which has close to 1100 members from 156 Member States. The objective of this network is to strengthen and enhance the capacities of national and regional laboratories in the surveillance of *Salmonella,* the other major food-borne pathogens and antimicrobial resistance in *Salmonella* and *Campylobacter* from humans, food and animals. More than 80 countries have provided data to the Country Databank on over 1.5 million human isolates and 360 000 isolates from non-human sources to help provide a global overview of the epidemiology of *Salmonella*. The External Quality Assurance System of WHO Global Salm-Surv is one of the world's largest annual proficiency tests with more than 150 laboratories participating worldwide.

3. SCOPE AND METHODOLOGY

3.1 This report describes those research projects within the UK funded by the MSFFG member organisations that investigate salmonellae serovars which cause food-borne, gastro-intestinal illness in humans. Projects which describe work on other *Salmonella* serovars are included where they provide additional information to the overall research effort on the organism. Additional projects on salmonellae are funded by the research councils (MRC and BBSRC) but have been excluded from the MSFFG database where they do not specifically include research on *Salmonella* in relation to food-borne illnesses. Similarly, research using other model organisms or systems and which may have broader relevance is not included. Post-graduate PhD studentships are also excluded from the MSFFG database.

3.2 At the time of writing this report, the member organisations of the MSFFG 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 Health Protection Agency (**HPA**), the Environment Agency (**EA**) the Medical Research Council (**MRC**) the Department of Agriculture and Rural Development, Northern Ireland (**DARD**), FSA Scotland, FSA Wales, FSA Northern Ireland, the Food Safety Protection Board (**FSPB**) and the Scottish Government[26]

3.3 Projects were identified for inclusion in the report by searching the MSFFG project database[27] using the search term "*Salmonella*" in the

[25] http://www.who.int/salmsurv/supported/en/
[26] Previously referred to as the Scottish Executive Environment and Rural Affairs Department (SEERAD).
[27] The MSFFG maintains a database (www.msffg.org.uk) containing information about research projects in the area of the microbiological safety of food that are funded by the members of the

search fields; title, key words or any component of the available text Projects that commenced from 2004 onwards were included, as were a small number of projects previously not captured in earlier reports, which were ongoing between 2004 and 2009. Further searches using the term "*Salmonella"* identified any additional relevant projects within the **BBSRC** oasis[28], the **Defra** R&D[29] databases and the **FSA** website[30].

4. SUMMARY DATA

4.1 This report is based on 80 projects identified from the database searches as defined above. The Funders of these projects are listed in the table below. A list of the projects is provided in Appendix 1.

Funder	**Number of projects funded**
Biotechnology and Biological Sciences Research Council (BBSRC)	47
Department for Environment, Food and Rural Affairs (Defra)	18
Food Standards Agency (FSA)	7
Medical Research Council (MRC)	5
Health Protection Agency (HPA)	2
Food Safety Promotion Board (FSPB)	1

Table 1: Allocation of *Salmonella* projects funded by the MSFFG

4.2 Research summaries are described in Sections 5 to 14. The initial sections summarise projects describing research into the biology of *Salmonella*. This includes studies into, for example, virulence factors present in the outer membrane of the bacteria, genomic studies and colonisation strategies. Further projects focus upon a number of areas including reduction and elimination strategies and antimicrobial resistance most often in the context of their association with the food chain. Surveillance techniques in animals and food production in the UK and EU are also discussed in a number of projects.

5. MOLECULAR BIOLOGY

5.1 A number of different mechanisms and structural components are involved in pathogenicity of salmonellae. These include the structures which enable the bacteria to attach and colonise the intestine and penetrate the mucosal wall, and those involved in the bacteria's ability to survive in a hostile host cell. In some *Salmonella* serovars these

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MSFFG. Members of the Group provide the project information from their respective project record systems.

[28] http://www.bbsrc.ac.uk/science/grants/index.html

[29] http://randd.defra.gov.uk/

[30] http://www.food.gov.uk/

mechanisms are likely to be host specific, whilst other mechanisms are found across serovars. An increase in the understanding of the mechanisms of pathogenicity at the structural, metabolic and molecular level may assist in developing strategies to minimise the effect of salmonellosis in animals and humans. The following sections describe those projects which are investigating active pathways during *Salmonella* infection.

5.2 Of relevance to the subfamilies which have chaperone:subunit adhesive organelles, including those of *Salmonella* was an examination of the structure of the stable polypeptide capsule by X-ray crystallography, mass spectrometry and atomic force microscopy which provided detail on folding and polymerisation (**BBSRC B16926**).

5.3 The secretion apparatus associated with flagellar assembly belongs to the type III secretion (T3S) family. The mechanism by which two flagellar T3S chaperones, FlgN and FliT, in *S. enterica* sense the progression of flagellar assembly, and regulate flagellar gene expression was investigated using characterised mutants of FlgN and FliT and measuring flagellar gene expression with respect to time (**BBSRC BB/D015855/1**).

5.4 *E. coli* contain CreBC, a regulator of the expression of genes whose products are involved in intermediary metabolism. To determine if the role of CreBC in *S.* Typhimurium is similar to that in *E. coli*, the *S.* Typhimurium LT2 cre regulon was analysed using microarray transcriptome analysis of CreBC over-active mutants versus wild-type LT2 (**BBSRC BB/C514266/1**).

5.5 *Salmonella* was used as a model of a system of iron storage and release from the molecule bacterioferritin (BFR). The mechanism of iron release was investigated to determine the extent of involvement of Bfd, (a ferredoxin associated with BFR). The specific roles of BFR and Bfd in *Salmonella* were tested using growth comparison experiments. (**BBSRC BB/D002435/1** and **BB/D001943/1**).

6. PATHOGENICITY

6.1 Pathogenicity is the ability of a microorganism to produce an infectious disease. Factors which are central to pathogenicity include the mechanisms and ease of colonisation of the host by the bacteria and the subsequent damage which occurs, and also the susceptibility and response of the host to the invading organism.

Virulence Factors

6.2 *Salmonella* serovars produce virulence factors; molecules that specifically cause disease, or that influence the host's function to allow the pathogen to thrive. Virulence factors can be structural proteins located in the outer

membrane of the bacteria, or may be a protein or metabolite involved in the metabolism of the bacteria.

6.3 Identification and characterisation of the virulence factors and associated genes which influence *Salmonella* pathogenesis, and the host response to infection, was carried out in cattle infected with wild type and mutant strains of *S.* Typhimiurium (**BBSRC BBSEI00001021**). Potential novel factors associated with virulence, colonisation pathogenicity islands and egg transmission were also identified for *S.* Enteritidis, *S.* Typhimurium and *S.* Typhi using a comparative genomic approach. The role of potential genes was investigated through the production of specific mutant strains that were tested using *in vitro* and *in vivo* infection models (**BBSRC BBSEI00001170**).

6.4 *S.* Dublin, but not *S.* Typhimurium, is able to translocate effectively and cause severe systemic salmonellosis in calves. The fates of conserved and serovar-specific tagged mutants of serovars Dublin and Typhimurium were tracked in calves using a bovine oral inoculation model in conjunction with an efferent lymphatic cannulation and signature- tagged mutagenesis (**BBSRC BBC50964X1**). A further investigation of oral inoculation of calves also used a signature-tagged transposon bank for *S. enterica* serovar Dublin to determine whether distinct virulence loci contribute to systemic translocation in calves (**BBSRC BBSEI00001179**).

Host susceptibility to infection

6.5 The relationship between host (pig and poultry) susceptibility to infectious diseases and colonisation as it relates to the pathogen, environment and husbandry systems was investigated in order to identify rational cost-effective control measures, to improve husbandry, and lead to a reduction in susceptibility to infections disease (**BBSRC BBSEF00042154**). The additional factors of commensal gut flora and genetic background in pigs were included in a separate project on host susceptibility; which also assessed the gene expression (particularly virulence genes), tissue distribution and host (poultry) immune responses to infection with *Salmonella* spp. (**BBSRC BBSEI00001160**).

Host–bacterial interactions

6.6 The interaction between *Salmonella* and a number of specific host cells including epithelium and macrophages have been investigated. The immune response of the host has also been examined by looking at the T cell population.

6.7 Surface structures derived from *S.* Typhimurium strain SL1344 were used to study the molecular basis of the interaction between the mucin layer of the gut epithelium, which is key for commensal microflora, and the surface structure of the bacterial cell wall (**BBSRC BBSEF00042240**). *Salmonella* virulence was also investigated by exploring the mechanisms

by which *Salmonella* grow inside host cells including epithelia (**MRC G9717183**).

6.8 Fluorescent Activated Cell Sorting (FACS) was used to purify sparse T cells or dendritic cells following their interaction with *Salmonella*. Subsequent functional studies on the cells included immunological, proteomic and transcriptomic analyses (**BBSRC BB/C511256/1**). The role of dendritic cells in directing the pathogen-specific T-cell response in a relevant human model was also a key objective investigated as part of a larger study on gut immunology and the interaction of food-borne pathogens (e.g. *Salmonella*) (**BBSRC BBSEF00041387**).

6.9 Macrophages and dendritic cells in *Salmonella* and *Mycobacterium* were used as paradigms of intracellular bacterial pathogens in genomic and bioinformatics programmes which were introduced to the Chinese Agricultural University with a China Partnership Award to study the host-pathogen interactions (**BBSRC BBSEI00001193**).

6.10 Screening samples for *Salmonella* was included as part of a study investigating the risk of *Campylobacter* infection from dogs. This provided added value to those studies in dogs which are already funded by Defra (**Defra OZ0612**).

Response of *Salmonella* to stress induced neuorendocrine molecules

6.11 There is increasing evidence to suggest that enteropathogens can sense host-produced neuroendocrine hormones (e.g. norepinephrine) released by the host under stress and respond to these environmental cues by activating growth and the expression of virulence genes. Evidence suggests that quorum sensing molecules may also play a role in the regulation of expression of virulence genes.

6.12 The ability of *S*. Typhi to sense and alter its physiology and pathogenicity in response to neuroendocrine (NE) stress hormones encountered during infection was investigated, together with the interactions between neuroendocrine hormones and the quorum sensing molecules autoinducer 2 and 3 (**MRC G0501449**). The influence of NE and tyramine and of alpha- and beta-adrenergic receptor antagonists, which have been found to inhibit the NE-induced response in *E. coli* O157:H7 and *S.* Choleraesuis *in vitro* were investigated in an adherence and invasion study of *E. coli* O157 and *S.* Typhimurium in calves and pigs using whole bacterial genome microarrays (**BBSRC BBC5180221**).

6.13 The effect of adrenergic receptor antagonists on EHEC and *S*. Typhimurium induced enteritis and colonisation were assessed and genes responsive to these agents, and associated with hormone signalling were identified (**BBSRC BBSEI00001195**).

Energy sources for metabolism

6.14 The role of sugars and fats as fuels for *Salmonella* during infection was investigated by blocking the manufacture of specific enzymes and transport proteins involved in the fuel breakdown pathways and seeing whether this reduces the ability of *Salmonella* to survive (**BBSRC BBS/E/F/00042073**). This relationship between the mechanisms used by *Salmonella* strains to obtain their energy and colonisation ability was also investigated with inbred lines of chickens **(BBSRC BBSEI00000990**).

6.15 *S.* Typhimurium genes which encode enzymes involved in central metabolism show differential expression during infection, which suggests that specific nutrients may sustain the growth of intracellular *S.* Typhimurium within the *Salmonella* Containing Vacuole (SCV) (a specialised acidic compartment inside macrophages). The invasion and colonisation ability of *S.* Typhimurium mutants with deletions of key metabolism genes tested this hypothesis in a mouse model (**BBSRC BBD0048101**).

Gene expression during the infection process

6.16 DNA microarray technology was a key method used by researchers to identify up and down regulation of bacterial genes during the infection process (**BBSRC BBSEF00041407**), in combination with Green Fluorescent Protein (GFP) (**BBSEF00042252** and **BBSEF00041208**) and to identify novel genes which are expressed during infection of murine macrophages by *S.* Typhimurium (**BBSRC BBC0055031**). In this latter study a number of gene deletions were introduced in the bacteria to compare survival and the host macrophage response in murine models.

6.17 The extent to which every non-essential gene of *S*. Typhimurium is required for intestinal colonisation of chickens, pigs and calves was determined using molecular genomic techniques (**BBSRC BBSEI00001255**).

6.18 A new method Transposon Mediated Differential Hybridisation (TMDH) in combination with oligonucleotide arrays was used to identify the function of virtually all transposable genes by simultaneously establishing the composition of mutant pools and location of insertion sites. The method has been validated in mice but required validation of the *S*. Typhimurium mutant libraries in chickens, pigs and calves (**BBSRC BBD0175561**, **BBD0179471** and **BBD0180801**).

6.19 *Salmonella* transcriptional networks involved in invasion and survival can be referred to as *Salmonella* extra and intracellular virulence gene expression programmes (STEX and STIN respectively). Unknown regulators of the STEX and STIN virulence gene programmes were identified (**BBSRC BBF00978X1**).

7. REDUCTION AND PREVENTION OF *SALMONELLA* IN FOOD ANIMALS

7.1 A number of strategies have been put in place to reduce the impact and prevalence of salmonellosis in the EU. Vaccines in poultry have undoubtedly been effective but still require further development, and there are a number of other projects focusing on non-vaccine methods of reducing *Salmonella* in food animals.

Vaccines

7.2 Vaccinations against *Salmonella* have become important in the control of *Salmonella*, with the majority of laying chicken flocks in the UK vaccinated against both *S.* Enteritidis and *S.* Typhimurium. There are a number of live vaccines available which can be given orally to create a gut-based immunity. Vaccines can be specific but in some instances vaccination will protect against other serovars in the same *Salmonella* group. Inactivated vaccines are given systemically but create an immunity which can be passively transferred to the offspring[31]. Vaccination is also used in the broiler chicken and breeding chicken industry sectors, in the latter, mostly in broiler breeders.

7.3 AtpA and trxA mutants of *S.* Typhimurium were highly attenuated and protective when used as live vaccines and were found to induce minimal inflammation in the spleens of infected animals. The mechanisms of attenuation, virulence, inflammation and protective immunity in invasive salmonellosis were examined for the potential of these mutants as vaccines (**BBSRC BB/E002943/1**). The role of complement and receptors for the Fc Fragment of Immunoglobin G (FcyR) in immunity to *Salmonella* was investigated (**MRC G0001245**).

7.4 A *Salmonella* vaccine which presents a specific *Campylobacter* protein (CjaA), to the chicken immune system was also found to protect against *Campylobacter* colonisation. The mechanisms of protection were investigated together with an assessment of colonisation prevention from other CjaA-type transporter proteins (**BBSRC BBD00019X1**) and (**BBSRC BBD0008661**).

7.5 Flagellin is the immunodominant T cell antigen recognised during *Salmonella* infections, yet it is dispensable for virulence and vaccination with soluble flagellin is not protective and can also impair the protection afforded by a live vaccine in part. Unravelling these mechanisms is key to developing successful vaccine regimes (**BBSRC BBF0227781**).

7.6 The suppression of adaptive, and cell mediated immunity at the onset of sexual maturity of laying hens was investigated using flow cytometry and immunocytochemistry to identify T and B cells. The study included

[31] http://www.intervet.co.uk/binaries/92_41146.pdf

assessment of the response to *Salmonella* infection and to vaccination with currently available vaccines during immunity suppression (**Defra OZ0327**).

Antimicrobial agents

7.7 One alternative to the use of conventional antimicrobials is the development of other agents (small chemical entities, proteins, predatory agents) that disable specific virulence factors of the microbial pathogen without affecting survival or growth.

7.8 The molecular mechanism which underlies the interaction between the SPI-1 encoded Type-III secretion system (TTSS-1) of the zoonotic pathogen *S. enterica,* and salicylanilides (a potential group of inhibitors) was investigated at the genomic level (**BBSRC BBD0106321**). The effect of mutational inactivation of *Salmonella* gene(s) to alleviate the inhibitory effect of salicylanilides was also examined (**BBSRC BBSEI00001241**).

7.9 Inhibitors of the multi drug-resistant efflux pumps may be a way of non-antimicrobial treatment. The ability of efflux pump inhibitors found in plant extracts to prevent avian colonisation by *Salmonella* by inhibition of the multi drug-resistant efflux pumps was investigated (**Defra ZM02206**).

7.10 An important role in defence against microbial infection involves defensins. Defensins are small proteins produced by cells of the immune system which bind to the bacterial cell membrane and form pores which in turn allow efflux of essential ions and nutrients. The mRNA levels of three new defensins were compared in cells of the immune system in the intestine and spleen of uninfected, infected and vaccinated chickens to investigate the role of the defensins to control intestinal colonisation by *Salmonella* and *Campylobacter*. Their direct effect of defensin protein on *Salmonella* and *Campylobacter* of various serovars was also investigated (**BBSRC BBSEI00001076**).

7.11 The antibacterial effect of antimicrobial peptides present in egg albumen upon *Salmonella* was investigated by studying the kinetics and mechanism of the interaction (**HPA 2004108**).

7.12 A study to investigate the ability of orally delivered predatory *Bdellovibrio* to kill *Salmonella* colonizing the intestines of chicks was extended to study the efficacy of *Bdellovibrio* in *Salmonella* treatment regimen and any longer term effect of *Bdellovibrio* on bird health and also to test the behaviour of *Bdellovibrio* mutant strains (**BBSRC BBG0030921**).

8. ANTIMICROBIAL RESISTANCE

8.1 The increase in prevalence of antimicrobial resistance in *Salmonella* is a concern for public health, as is the increasing number of classes of antimicrobial agents involved. Complex groups of genes may encode for

resistance with little cost to the bacterium. Antimicrobial resistant *Salmonella* have a selective advantage when transferred to the gut ecosystem of animals or humans treated with antimicrobials and therefore emergence of resistance and increased transmission are often linked (Riemann & Cliver, 2006).

8.2 Much of the research described here in the area of antimicrobial resistance has focussed on the identification and further characterisation of antibiotic resistance genes using genomic studies.

8.3 A range of genomic based tests (PCR, microarrays) were used for the identification and characterisation of specific antimicrobial resistance genes, plasmids and/or integrons in a) *S. enterica* with resistance to third-generation cephalosporins and mutations conferring resistance to quinolone antibiotics (**Defra VM02136**), b) fluroquinolone resistant isolates of *S*. Paratyphi A (**MRC G0600805**) and c) strains of *S.* Typhimurium phage type U288, multi drug- resistant *S.* Typhimurium DTs 104 and 193 and multi drug-resistant *S.* Paratyphi B variant Java (**Defra VM02205**).

8.4 An investigation into the resistance to the antimicrobial Triclosan, which acts as an inhibitor of fatty acid biosynthesis by binding to FabI protein, in *S.* Typhimurium involved identification of the genes involved, and microarray analysis of FabI using mutants to identify differences in gene expression between strains presenting with different levels of resistance (**BBSRC BB/D020476/1**).

8.5 The mechanism behind the development of resistance to antimicrobial agents in *S*. Typhimurium following exposure to disinfectants was investigated together with studies to determine whether multi drug resistance to biocide stress can occur independently of loss of virulence (**Defra OD2029**).

8.6 The relationship behind multiple antibiotic resistance (MAR) and chromosomally encoded efflux pumps was investigated in non-typhoidal *S. enterica*. Gene expression of MAR mutants as compared with the parent strains and cloned MAR from two mutants were used for further gene characterisation (**MRC G0501415**).

8.7 The use and effectiveness of pre, pro and symbiotics in food producing animals for reduction in the carriage of antimicrobial resistance against bacteria including multi drug-resistant *S*. Typhimurium was investigated by using antimicrobial resistance gene probes *in vitro* (**Defra VM02203**).

8.8 The focus of antimicrobial resistance in bacteria has been on the administration of antimicrobial agents to food animals particularly when given prophylatically and/or as growth promoters. To assess whether pet animals may be a further source of antimicrobial resistant bacteria and the risks that this brings, a study into the prevalence of pathogenic

antibiotic-resistant bacteria, e.g. *Campylobacter* and *Salmonella* was undertaken in cats, dogs and rabbits (**Defra OD2026**).

8.9 A comparative risk assessment was formulated on the likelihood of the development of antimicrobial resistance in zoonotic salmonellas in relation to; the veterinary use of antimicrobials in the UK, and such usage in countries without the UK which regularly export animal food products into the UK (**Defra ZM02209**).

9. POULTRY AND EGG PRODUCTION

9.1 The most common serovars of *Salmonella* associated with poultry and eggs are *S.* Enteritidis and *S.* Typhimurium. The European Food Safety Authority (EFSA) reported the EU prevalence estimate of *Salmonella*-positive flocks at 23.7%, with a range of 0% to 68.2%[32]. In the UK prevalence is lower than the mean EU prevalence in both laying hens (7.9%) and in UK produced eggs (see sections 1.16-1.17). However, since eggs and egg products are considered important sources of salmonellosis in food-borne outbreaks and control of *Salmonella* in chicken flocks laying eggs for human consumption is a requirement according to the Zoonoses Regulation, there is a need to continue to monitor and control *Salmonella* in the UK poultry and egg industries. Monitoring and investigation of any positives is a legal requirement under the Zoonoses Directive and Regulation 2003/99/EC.

9.2 A validation procedure for elimination of *S*. Enteritidis (SE)/*S*. Typhimurium (ST) from commercial laying flocks was described following an assessment of the level of contamination and a characterisation of the public health risks (**Defra OZ0332**).

9.3 New *S. enterica* phage types (SE PTs) and other potentially egg-associated serovars were investigated in relation to the effectiveness of current vaccination strategies and outbreaks in hens and eggs. A comparative genomic study between serovars was performed together with an assessment of the risks posed by new SE PTs in their ability to survive and grow and thus alter the shelf life of eggs (**Defra OZ0326**).

9.4 The molecular bases for the tropism of *S*. Enteritidis for hen reproductive tissues with a focus on the lipo-polysaccaride surface structure was investigated to determine the molecular bases underlying the survival of SE in avian macrophages (**FSA B15027 and B15028**), in a co-sponsored project together with projects **BBSRC BBF0079731** and **BBF0072211**.

9.5 A qualitative risk assessment for *Salmonella* in shell eggs and processed eggs in Ireland was developed (**FSPB 845**).

[32] http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178620761745.htm

9.6 An analysis of egg contamination by *Salmonella* and other pathogens after moving laying hens to enriched cages and alternative housing systems was carried out in order to predict the potential risk to the consumer. In addition, genes were identified which were involved in *Salmonella* survival, infection, and antibiotic drug resistance (**BBSRC BBS/E/F/00042300**).

9.7 Changes in the immune system in maturing hens, for example the levels and function and proliferation of T and B lymphocytes and the expression of key cytokines, were measured following a) *Salmonella* infection b) vaccination and c) during point-of-lay, when it is thought there may be a suppression of immunity that may compromise protection against *S.* Enteritidis. A greater understanding of the immune system could lead to modifications in vaccine strategy for a more effective protection (**BBSRC BBD0075421**).

9.8 The ability to induce innate resistance to colonisation by *Salmonella* by delivering a live attenuated *Salmonella* vaccine to newly hatched chicks was investigated by introducing attenuating mutations and assessing the host cell responses in broilers under experimental and simulated field conditions. Other strategies for elimination of *Salmonella* which included use of inducible bacterial suicide genes and host defensin production were also assessed (**BBSRC BBSEI00001109**).

9.9 Genes were identified that controlled variation in innate immune responses and the extent of genetic variability in loci that influence the innate immune response of the chicken was defined and related to resistance/susceptibility to *Salmonella* and *Campylobacter*. This provided new opportunities for selective breeding of commercial broilers for improved resistance to enteric bacteria (**BBSRC BBSEI00001227**).

9.10 An investigation into the extent of genetic variability of loci that influence the innate immune response of chickens and therefore resistance /susceptibility to *Salmonella* (and *Campylobacter*) was performed. This study aimed to show poultry breeders the potential of selective breeding of commercial broilers with improved innate resistance to enteric disease and hence improved nutrient capture and food safety (**Defra LK0665**).

9.11 A review was undertaken to understand the contribution made by *Salmonella* present in animal feed to overall *Salmonella* infection of poultry and contamination of poultry houses and products (**Defra OZ0329**).

9.12 The sourcing and handling of raw eggs in businesses associated with a food-borne disease outbreak of *S.* Enteritidis was included as part of a larger study investigating managerial and operational risk factors associated with food-borne disease outbreaks in the catering industry (**FSA B13004**).

10. MEAT PRODUCTION

10.1 A review of current and potential alternative pig slaughter processes was carried out in combination with a practical and economic assessment to identify the highest potential to reduce contamination in pig carcasses, to meet the EU 2010 target of 50% reduction in *Salmonella* (**FSA M01038** and **M01040**).

10.2 The level of success of implementation of disease control on live-stock farms is influenced by the tools or strategies used to promote interventions and encourage uptake. The impact of establishing "demonstration farms" within the English pig industry to disseminate information on disease control in pig herds were investigated (**Defra OZ0148**).

10.3 A farm-to-fork quantitative microbiological risk assessment (QMRA) in pigs evaluated the level of exposure and the subsequent risk to human health due to *S.* Typhimurium (**Defra OZ0331**).

11. FRUIT AND VEGETABLES

11.1 Although contaminated foods are often of animal origin, such as beef, pork, poultry, milk, or eggs, other food, for example vegetables and fruits, may harbour *Salmonella* from contamination with fertilizers of faecal origin, or if washed with polluted water or from cross-contamination from practices within the food production industry.

11.2 The mechanisms used by *S. enterica* and EHEC to attach to salad leaves were investigated to determine the level and pattern of adhesion and to visualize potential adhesion factors. Host factors that contribute to leaf susceptibility to colonisation were also examined using genomic approaches (**BBSRC BBG0135431**).

11.3 The interactions between *Salmonella* and plant tissue was investigated in order to understand the mechanisms of survival and growth (**BBSRC BBSEF00042282**); and also why attachment processes provide an environment for the bacteria that prevents a completely successful commercial decontamination (**BBSRC BBSEF00041966**). The information was then used to develop intervention strategies to prevent the colonisation of ready-to-eat fruit and vegetables by bacteria together with processes for bacterial decontamination.

12. EPIDEMIOLOGY

12.1 Epidemiology is the study of the distribution and determinants of diseases within populations. Epidemiology studies are used for a number of descriptive purposes; surveillance of the occurrence of the disease, study of disease progression, identification of prognostic factors and evaluation of treatments (Greenberg *et al*., 2005).

12.2 A report on the Department of Health funded IID (Infectious Intestinal Disease) Study in England by the FSA in 2000 defined disease burden, and calibrated a national surveillance system, (an estimation of the factor by which the number of cases of infection with specified pathogens needed to be multiplied to establish the actual number of infections in the community). Since this original study was undertaken, several structural changes have occurred in the national surveillance. To repopulate the calibration with contemporary data a second IID study (**FSA B18021**) was commissioned to estimate prospectively the burden and causative agents of IID in the population and compared the results with previous national surveillance data. An archive of nucleic acid extracts were also generated, maintained and administered from IID archived gastrointestinal pathogen positive faecal specimens. Assessment of the extraction technique was performed by using PCR based techniques to detect selected pathogenic agents (**FSA B14004**).

12.3 A UK case control study was performed to investigate if there are a specific set of risk factors for *Campylobacter* and *Salmonella* infection in infants aged less than one year (**HPA 2004132**).

13. SURVEILLANCE TECHNIQUES

13.1 It is not common practice for the MSFFG to consider projects involved in surveillance. The projects summarised below are included as they describe some of the broader aspect of suitability of new analytical methods (e.g. PCR), calculation of risk factors and development of sampling protocols.

13.2 A number of surveillance studies across the UK and Europe have been performed which together provide a substantial amount of data on prevalence of *Salmonella*. In addition there have been a number of projects which have looked at the robustness of those protocols which are used during identification and surveillance studies.

13.3 An epidemiological analysis generated risk factor data from farms selected for an EU Breeding Pig Survey and identified optimum food chain control strategies for the control of *Salmonella* in pigs and the reduction in human salmonellosis (**Defra OZ0330**).

13.4 Two projects have been ongoing to assist the egg industry (**Defra OZ0325)** and the turkey industry (**Defra OZ0328**) with achieving the EU

prevalence reduction targets outlined in EU Zoonoses Legislation (Directive 2003/99, Regulation 2160/2003 and relevant species specific EU legislation). Researchers verified a number of protocols used for *Salmonella* monitoring (e.g. sensitivity, group sizes, sampling methods), control (e.g. time of delivery of vaccines methods for improving vaccination response), and risk reduction (adult hygiene, other vectors).

13.5 Epidemiological, risk-based and molecular data were gathered for new and emerging *Salmonella* serovars in farmed animal species. Resulting datasets were used to improve evidence-based sampling protocols, risk and spatial analytical models and to develop statistical theory, code and geographical information system visualisation (**Defra OZ0324**).

14. OTHER PROJECTS

14.1 The database search revealed six projects (listed in Appendix 2) which have recently been captured in other reports from the Microbiological Safety of Food Funders Group. The relevant reports are 1) *UK publicly funded research relating to risk assessment and the microbiological safety of food*.[33] 2) *UK publicly funded research on microbial antibiotic resistance in relation to the safety of food*[34] and 3) *UK publicly funded research relating to food preparation practices and behaviour in relation to the microbiological safety of food*[35]

15. GAPS IN CURRENT MSFFG FUNDED RESEARCH

15.1 Genomic information is available for a number of *Salmonella* serovars and is utilised by a large number of projects. Investigations include those assessing the behaviour and growth of *Salmonella* mutants devoid of genes of interest, and identification of genes involved in *Salmonella* infection, for example, pathogenicity or antimicrobial resistance. Other areas of research include; effectiveness of pathogenicity, responses to environmental cues and bacterial/host interactions, for example the immune response to infection by the host. Projects described in this update often use microarray technologies and PCR for the identification of genes and proteins present within *Salmonella*; such tools were identified in the 2005 *Salmonella* MSFFG report as likely to be used in future projects. As observed in this report, studies of the genetics and physiology of *Salmonella* continue to encompass a broad field of research into the fundamental biology of the organism.

[33] http://www.food.gov.uk/science/research/researchinfo/foodborneillness/microfunders/msffg/msffgriskassessmentreport

[34] http://www.msffg.org.uk/reports/antibioticresistance.html

[35] http://www.food.gov.uk/science/research/researchinfo/foodborneillness/microfunders/msffg/msffgjan09

15.2 Investigations of the bacterial genome and proteome produce large amounts of data which require analysis by bioinformatics specialists. Such aspects of data analysis are not commonly described within the project's core deliverables and it would be prudent to highlight that such specialists should be involved. While large amounts of data have been generated by projects there is a gap in the provision of an overarching central repository for the data which could perhaps identify any overlaps and common themes. While a number of projects now describe the study of *Salmonella* in appropriate models, commonly cattle or pigs, a gap still exists in the understanding of the relationship between data from *in vitro* models and findings observed in cattle, poultry and man.

15.3. This gap can be further extended to translation of the data observed in the genetic and physiological studies to furthering our understanding of *Salmonella* food poisoning; causes, reduction, prevention and antimicrobial drug resistance – described as translational research. Further information on many aspects of translational research may be found in the report *Systems biology: a vision for engineering and medicine* published by the Academy of Medical Sciences and The Royal Academy of Engineering in 2007. This report highlights systems biology as a groundbreaking new approach to scientific research and also calls for more support for research at the intersection of engineering and biology to ensure the UK remains competitive.

15.4 In the 2005 MSFFG report it was suggested that there was little research to address the role of phage integration in the epidemiology, survival and pathogenicity of *Salmonella*. The shift in recent years from less PT4 *S*. Enteritidis to more non-PT4 *S*. Enteritidis underlines the continuing need for such studies particularly on identification, integration, persistence of other phage types and their effect on pathogenicity and virulence attributes.

15.5 Current research into development of antimicrobial resistance in *Salmonella* has focused upon identification of resistance genes. Thus there is now a need to use this genomic data and focus beyond gene identification to understanding and developing strategies to minimise development of resistance and also how changes in treatment can affect the bacterial genome.

15.6 A number of projects describe work on vaccines. While there has been a decrease in the incidence of *Salmonella* in the broiler and egg industries, work on effectiveness of vaccination has continued. The success of vaccination in broiler chickens suggests that vaccines may be a route to reduction of *Salmonella* in meat. Vaccine approaches have a number of caveats. There is a need for continued development of effective vaccines, and also research on the effectiveness of vaccines within tissue during meat production process. While, the focus of vaccine development has been on the two main serovars, *S.* Typhimurium and *S.* Enteriditis, it may be prudent to not dismiss the need for new vaccines should the impact of other food-related *Salmonella* serovars change in the future.

16. CONCLUSIONS

16.1 This report demonstrates that there is continued government support for research on *Salmonella* in the UK and Europe, the results of which are likely to deliver a comprehensive understanding of the pathogenicity of these organisms, and also a reduction and prevention of infection with *Salmonella* in the context of food-borne illness.

16.2 One of the key advances has been the use of molecular biology as a conduit to understand *Salmonella* at the genetic level. Many research projects have utilised molecular methods to explore the genetic basis of pathogenicity, antimicrobial resistance and vaccine research. In addition many surveillance projects, including those which are more global in nature are harnessing molecular biology techniques such as PCR as a universal method for serovar identification. The consequence of this increase in use of molecular tools is the generation of large amounts of data that require careful mining and interpretation. Researchers should be cautious with respect to the level of relevance that genetic information that is obtained plays in the *in vivo* situation.

16.3 In the previous MSFFG *Salmonella* report it was suggested that a gap exists between the knowledge developed through laboratory-based studies and its application to real-life situations “in the field”. While a number of projects described in this update have used relevant *in vivo* models, which go some way to address this concern, there are still many studies where the applicability to “real-life” *Salmonella* infections is still to be made.

16.4 The reduction in incidence of reported *Salmonella* cases in the UK is likely to be indicative of the large amounts of work to identify and reduce risk factors involved in *Salmonella* infection, and the implementation of changes in working practices in the poultry meat and egg industries. This in part was precipitated in 2003 by the introduction of two EU regulations; (EC) 2160/2003[36] *on the control of Salmonella and other specified food-borne zoonotic agents,* and the EU Zoonoses directive 2003/99/EC concerns the monitoring of oonoses and zoonotic agents.

16.5 Studies which aim to bridge the gap between the fundamental studies of *Salmonella* biology, and an understanding of the control of behaviour of the bacteria in food animals and during meat production are likely to be of importance for moving forward in investigating this food-borne zoonoses in order to reduce its impact upon the consumer. Current research also focuses upon improvements in vaccines and vaccination strategies and immune responses in poultry and continued surveillance is also featured.

[36] http://eur-lex.europa.eu/LexUriServ/site/en/oj/2003/l_325/l_32520031212en00010015.pdf

16.6 The occurrence of salmonellosis in the UK is still important. There is therefore a continuing need for animal and food surveillance, epidemiology studies and studies on consumer practices and to relate such data to identify patterns in the infection routes. Unification of methods for surveillance and identification of antimicrobial resistant genes is under development and techniques such as PCR and use of microarrays for *Salmonella* identification appear more common.

REFERENCES

ACMSF (1999) Report on microbial antibiotic resistance in relation to food safety. The Stationery Office, London ISBN 0 11 322283 1

Greenberg Raymond S, Daniels Stephen R, Flanders W Dana, William Eley John (2005), Medical Epidemiology, McGraw-Hill Professional

Kauffmann F (1961) , The species definition in the *Enterobacteriaceae.* International Bulletin Bacteriol. Nomencl. Taxon. **11**:5-6

Riemann Hans and Cliver Dean O. (2006), Food-borne infections and intoxications, Academic Press

Systems biology: a vision for engineering and medicine (2007)
A report from a working group of The Academy of Medical Sciences and The Royal Academy of Engineering, ISBN No. 1-904401-13-5

GLOSSARY

Antibiotic
A substance produced by or derived from a microorganism, which selectively destroys or inhibits the growth of other microorganisms. Because compounds such as sulphonamides and quinolones are synthesised chemically, they are not strictly speaking antibiotics. However, in practice the term “antibiotic” often encompasses such agents (ACMSF (1999)).

Antimicrobial agent
A substance which kills microorganisms or suppresses their replication or growth.

Antimicrobial resistance
The ability of a microorganism to resist the effects of an antimicrobial agent. May be an intrinsic characteristic or acquired by selection for mutation or by acquisition of a resistance gene from other microorganisms.

B cells
Lymphocytes which are involved in the host immune response. Their principal functions are to make antibodies against antigens, perform the role of Antigen Presenting Cells.

Bacteriophage, abbreviation phage
Virus that infects a bacterium.

FACS Fluorescent Activated Cell Sorting
A method in which populations of cells can be separated from a heterogenous mixture using flow cytometry to isolate cells with different light scattering and fluorescent characteristics.

Genome, genomics, gene expression
The **genome** encompasses both the genes and the non-coding sequences of the DNA of one set of chromosomes. **Genomics** is the study of the genomes of organisms and includes determining the DNA sequence of organisms and genetic mapping. Gene expression is the process by which the information contained within a gene is used to produce ribonucleic acid (RNA) which functions as a template for protein production or as a molecule involved in regulation of the genome.

Genomic Island (GI)
A previously mobile element, which has subsequently become incorporated and fixed into the bacteria genome. GI’s are characterised by their large size(>10 Kb), often have a different base content compared with the rest of the genome, and also carry fragments of other mobile elements such as phages and plasmids. Some genomic islands can excise themselves spontaneously from the chromosome and can be transferred to other suitable recipients.

GFP (Green fluorescent protein)
A protein which fluoresces when exposed to light and can be synthesized by a cell following insertion of the GFP gene into its genome. GFP is often inserted alongside a gene of interest. When transcription occurs to both genes, GFP is produced, can be visualized and reports that the gene of interest has also been transcribed.

Integrons
A DNA element that can convert exogenous gene cassettes found in plasmids, chromosomes and transposons into functional genes by ensuring their correct expression. These DNA elements contain hundreds of accessory genes and constitute a significant fraction of the genomes of many bacterial species.

Microarray
A multiplex technology consisting of an arrayed series of thousands of microscopic spots called features. Features can be for example, a short section of a gene or other DNA element that are used as probes to which related cDNA or cRNA sample will anneal. Annealing is usually detected and quantified by fluorescence-based detection of fluorophore-labeled targets.

PCR (Polymerase Chain Reaction)
A widely-used molecular method used to generate multiple copies (amplification) of a target DNA sequence.

RT-PCR (Reverse Transcriptase Polymerase Chain Reaction)
A molecular method in which an mRNA target sequence is converted into a complementary DNA strand.

Plasmids
A DNA molecule that occurs separately from chromosomal DNA and is capable of replicating independently of the chromosomal DNA.

Proteome
The set of proteins expressed by the genome of an organism, or cell.

Quorum sensing molecule
A molecule which is released by one or more bacteria of a colony to trigger coordinated behaviour, e.g. gene expression, among the population as a whole.

Serovars, serovar
Subdivisions of a (bacterial) species identified by their antigenic characteristics.

Translational research (translation, translate)
The process of the bidirectional transfer of knowledge between basic work (in the laboratory and elsewhere) with that of the person, in health or disease[37]. In

[37] Medical Research Council Workshop: Accelerating the Translation of medical research (2007). http://www.mrc.ac.uk/Utilities/Documentrecord/index.htm?d=MRC003642

the context of this report translational may be described as transfer of knowledge from the organism on the bench to within the food industry.

Translocation
The movement of bacteria between and across tissues membranes e.g. from the intestines to distal organs.

Transcriptome
The set of all messenger RNA (mRNA) molecules, or "transcripts," produced in a cell. Unlike the genome, the transcriptome can vary with external environmental conditions.

Type III secretion
A specialised secretion system found in many Gram-negative bacterial pathogens, which can deliver virulence proteins directly into host cells.

T cells
Lymphocytes which are involved in the immune response. Their principal functions are in cell-mediated immunity. Unlike B cells they possess a receptor on their cell surface called T cell receptors. Several different subsets of T cells have been discovered, each with a distinct function.

STM (Signature Tagged Mutagenesis)
A technique where genes are altered with random insertions of small mobile DNA sequences (which are themselves tagged with a sequence for identification) into a genome. When the genes are transcribed and translated the protein structures are altered and looses function but can be traced via the tag. This method can be used as a means of identifying virulence genes in bacteria.

Virulotyping
The typing of bacteria based on their virulence factors using microarrays.

APPENDIX 1 LIST OF PROJECTS DESCRIBED IN THIS REPORT

Project Code	Title	Funder	Start Date	End Date
B16926	Chaperone dependent assembly and structure of a bacterial polypeptide capsule	BBSRC	01-May-02	30-Apr-05
BB/C511256/1	Cell sorting for functional and post-genomic analysis of rare immune and infected cells	BBSRC	01-Oct-05	30-Sep-06
BB/C514266/1	Transcriptional networking through the Cre regulons of *Escherichia coli* and *Salmonella* Typhimurium	BBSRC	01-Jun-05	31-May-08
BB/D001943/1	Iron mobilisation in the bacterial cell	BBSRC	01-Jul-06	30-Jun-09
BB/D002435/1	Iron mobilisation in the bacterial cell	BBSRC	09-Jan-06	08-Jan-09
BB/D015855/1	Uncoupling flagellar gene expression from flagellar assembly	BBSRC	01-Aug-06	31-Jul-09
BB/D020476/1	Characterisation of triclosan resistance in *Salmonella enterica* serovar Typhimurium.	BBSRC	01-Jan-07	31-Dec-11
BB/E002943/1	Characterisation of the pathogenesis and immunogenicity of two novel attenuated mutants of *Salmonella* Typhimurium	BBSRC	02-Jan-07	01-Jan-10
BBC0055031	Adaptation of intracellular *Salmonella enterica* serovar Typhimurium to survival in the host environment	BBSRC	01-Mar-05	31-Aug-08
BBC50964X1	Characterisation of the molecular basis of systemic salmonellosis in calves	BBSRC	01-Jun-05	31-May-08
BBC5180221	Influence of the neuroendocrine stress hormones on the carriage and virulence of zoonotic bacterial pathogens in farm animals	BBSRC	01-Apr-05	30-Nov-09

Project Code	Title	Funder	Start Date	End Date
BBD00019X1	Dissection of protective responses to heterologous *Campylobacter* vaccines	BBSRC	19-Jun-06	18-Jun-09
BBD0008661	Dissection of protective responses to heterologous *Campylobacter* vaccines	BBSRC	01-Jul-06	30-Jun-09
BBD0048101	The role of central metabolism in the successful infection of macrophages and mice by *Salmonella* Typhimurium	BBSRC	01-Jul-06	30-Jun-09
BBD0075421	Effect of immunosuppression associated with point-of-lay on *Salmonella* infection and immunity in laying hens	BBSRC	03-Jul-06	02-Jul-09
BBD0106321	Inhibition of bacterial Type III secretion by salicylanilides	BBSRC	12-Oct-06	11-Oct-09
BBD0175561	Global assignment of the function of *Salmonella* genes in livestock	BBSRC	01-Oct-06	30-Sep-09
BBD0179471	Global assignment of the function of *Salmonella* genes in livestock	BBSRC	01-Sep-06	31-Aug-09
BBD0180801	Global assignment of the function of *Salmonella* genes in livestock	BBSRC	01-May-06	30-Apr-09
BBF0072211	Molecular mechanism underlying the interaction of *Salmonella* Enteritidis with the hen oviduct and survival in eggs	BBSRC	01-Sep-08	31-Aug-11
BBF0079731	Molecular mechanism underlying the interaction of *Salmonella* Enteritidis with the hen oviduct and survival in eggs	BBSRC	01-Mar-08	28- Feb-11
BBF00978X1	Regulation of the ppGpp-dependent virulence gene programmes of *S.* Typhimurium	BBSRC	11-Nov-08	10-Nov-11
BBF0227781	Activities of *Salmonella* flagelin: FliCing immunity on and off from Th1 to Th2	BBSRC	19-Dec-08	18-Dec-11
BBG0030921	Fromm curiosity to curative -developing *Bdellovibrio* as living antibiotics between farm and fork	BBSRC	01-Oct-08	30-Sep-11

Project Code	Title	Funder	Start Date	End Date
BBG0135431	Bacterial and plant factors that influence adhesion of enterohaemorrhagic *E. coli* and *Salmonella enterica* to salad leaves	BBSRC	23-Feb-09	22-Feb-12
BBS/E/F/00042073	The role of central metabolism in the successful infection of macrophages by *Salmonella* Typhimurium	BBSRC	01-Jul-06	30-Jun-09
BBS/E/F/00042300	Analysis and control of egg contamination by *Salmonella* and other pathogens after moving laying hens to enriched cages and alternative housing systems	BBSRC	01-Oct-06	30-Sep-09
BBSEF00041208	Molecular microbiology of *Salmonella* Typhimurium and *E. coli*	BBSRC	01-Apr-00	31-Mar-05
BBSEF00041387	Gut immunology	BBSRC	01-Apr-01	31-Mar-05
BBSEF00041407	Bacterial functional genomics: Dissemination of cutting-edge technology (BACFUN)	BBSRC	31-Jan-02	09-Apr-06
BBSEF00041966	Factors affecting the attachment of bacteria to, and their detachment from, prepared fruit and vegetable tissues	BBSRC	01-Oct-05	30-Sep-07
BBSEF00042154	Animal susceptibility to infection and disease: do husbandry systems and welfare drive microbial colonisation and immune development	BBSRC	01-Jan-05	31-Dec-07
BBSEF00042240	The *in vitro* assembly and interactions of *Salmonella* surface structures	BBSRC	01-Apr-06	01-Apr-07
BBSEF00042252	Molecular microbiology of *Salmonella*	BBSRC	01-Apr-05	01-Apr-07
BBSEF00042282	Molecular biology of *Salmonella* attachment to plant tissues	BBSRC	01-Apr-05	01-Apr-07
BBSEI00000990	Bacterial and host genes in *Salmonella* colonisation in poultry	BBSRC	01-Jul-02	30-Jun-05
BBSEI00001021	*Salmonella* pathogenesis in cattle and pigs	BBSRC	01-Jul-02	30-Sep-06

Project Code	Title	Funder	Start Date	End Date
BBSEI00001076	Intestinal defensins and their role in controlling avian *Salmonella* and *Campylobacter* infection	BBSRC	01-Oct-03	30-Sep-06
BBSEI00001109	*Salmonella*-free broilers by live vaccine-induced innate resistance to colonisation and invasion and novel methods to eliminate vaccine and field strains	BBSRC	01-Feb-04	31-Jul-07
BBSEI00001160	Animal susceptibility to infection and disease: do husbandry and welfare drive microbial colonisation and immune development?	BBSRC	01-Jun-04	31-May-09
BBSEI00001170	Virulence and colonisation factors of *Salmonella* Enteritidis	BBSRC	01-Oct-04	30-Sep-07
BBSEI00001179	Characterisation of the molecular basis of systemic salmonellosis in calves	BBSRC	01-Mar-05	28-Feb-08
BBSEI00001193	Genomic and post genomics of *Salmonella* and *Mycobacterium* as paradigms of intracellular pathogens	BBSRC	01-Mar-05	28-Feb-06
BBSEI00001195	Influence of neuroendocrine stress hormones on the carriage and virulence of zoonotic bacterial pathogens in farm animals	BBSRC	01-Apr-05	31-Mar-08
BBSEI00001227	Improving the gut health and nutrient capture of broiler chickens through selection for innate immune function	BBSRC	01-Jan-06	31-Dec-08
BBSEI00001241	Inhibition of bacterial Type III secretion by salicylanilides	BBSRC	12-Oct-06	11-Nov-09
BBSEI00001255	Global assignment of the function of *Salmonella* genes in livestock	BBSRC	01-Oct-06	30-Sep-09
LK0665	Improving gut health and nutrient capture of broiler chickens through selection for innate immune function	Defra	01-Jan-06	31-Dec-08
OD2026	The clinical treatment of pet dogs and antibiotic resistance in commensal and potentially pathogenic bacteria	Defra	01-Jun-07	31-May-10

Project Code	Title	Funder	Start Date	End Date
OD2029	Evolution of multidrug resistance in *Salmonella* enterica serovar Typhimurium as a result of biocide exposure	Defra	01-Mar-09	01-Mar-12
OZ0148	Tools in Knowledge transfer and adoption of disease control among farmers, the effect of demonstration among pig farmers	Defra	Aug-08	Mar-11
OZ0324	New and emerging *Salmonella* serovars; epidemiological, risk-based and molecular approaches to their identification and control	Defra	01-Jan-05	01-Jan-09
OZ0325	Monitoring, Control and Education Package to assist the Egg Industry with *Salmonella* reduction and achieving EU targets.	Defra	01-Apr-06	31-Mar-09
OZ0326	Managing the challenges to the egg industry posed by new *S*. Enteritidis phage types	Defra	01-Apr-06	31-Mar-09
OZ0327	Effect of immunosuppression associated with point-of-lay on *Salmonella* infection and immunity in laying hens	Defra	01-Apr-06	31/03/2009
OZ0328	A monitoring, control and education package to assist the Turkey industry with reduction of *Salmonella* and antimicrobial resistance and achieving EU targets	Defra	01-Jan-07	01-Jan-10
OZ0329	A comprehensive review of the treatment of feeding stuffs related to the control of *Salmonella* in laying flocks	Defra	Aug-07	May-08
OZ0330	A report on the further analysis of the data collected on the UK breeding pig *Salmonella* survey to obtain information of the risk factors of transmission in pigs	Defra	Apr-08	Jun-11
OZ0331	Qualitative microbiological risk assessment on *Salmonella* in slaughter and breeder pigs	Defra	Apr-08	Mar-09

Project Code	Title	Funder	Start Date	End Date
OZ0332	*S.* Enteriditis and *S.* Typhimurium in egg production.: Elimination from flocks and characterisation of public health risks	Defra	Apr-09	Mar-12
VM02136	Development of rapid response gene profiling for identification of antimicrobial resistance genes in enterobacteria from food animals and humans	Defra	01-Mar-03	31-May-06
VM02203	Interventions to reduce the carriage of antimicrobial resistance in food producing animals	Defra	01-Apr-06	30-Nov-07
VM02205	Genoprofiling of multiresistant *Salmonella enterica*	Defra	01-Jun-06	31-May-07
VM02206	Do efflux pump inhibitors prevent avian colonisation by *Salmonella* and *Campylobacter*	Defra	01-Jun-08	31-Aug-09
VM02209	What is the relationship between the veterinary use of antimicrobials and drug resistance in zoonotic pathogens	Defra	01-Jan-09	31-Dec-09
MVN-WP04	Development of a linked molecular surveillance database system for food-borne infections (PulseNet Europe)	EU MED-VET-NET	01-Sep-04	28-Feb-07
MVN-WP09	The human health implication of emerging resistance to beta-lactam antibiotics in *Salmonella* and other Enterobacteriaceae from food animals	EU MED-VET-NET	01-Sep-06	28-Feb-06
MVN-WP10	Validation and standardization of PCR-based methods for detection and quantitative risk assessment of food-borne pathogens (Food-PCR 2)	EU MED-VET-NET	01-Sep-04	28-Feb-06
MVN-WP21	Molecular epidemiology of *Salmonella* Genomic Island 1 (SGI)	EU MED-VET-NET	01-Mar-06	
MVN-WP26	Virulotyping of new and emerging *Salmonella* and VTEC	EU MED-VET-NET	01-Mar-06	
MVN-WP28	Methods of attributing human *Salmonella* and *Campylobacter* infections with different animals, food and environmental source	EU MED-VET-NET	01-Mar-06	

Project Code	Title	Funder	Start Date	End Date
MVN-WP33	Early host responses to *Salmonella* and *Campylobacter*	EU MED-VET-NET	01-Mar-06	
B13004	Management Risk Factors resulting in food-borne disease outbreaks in the catering industry – a case control study	FSA	01-Oct-01	31-Mar-05
B14004	Generation of an archive of extracted nucleic acid for the IID archived faecal specimens	FSA	01-Jan-03	31-Dec-07
B15027	Molecular Mechanisms underlying the interaction of *Salmonella* Enteritidis with the Hen Oviduct and Survival in Eggs	FSA	01-Sep-08	31-Aug-11
B15028	Molecular Mechanisms underlying the interaction of *Salmonella* Enteritidis with the Hen Oviduct and Survival in Eggs	FSA	01-Mar-08	28-Feb-11
B18021	The second study of infectious disease in the community - determining disease burden and calibrating national surveillance data in the United Kingdom	FSA	01-Apr-06	31-May-10
M01038	Reduction of *Salmonella* contamination of pig meat.	FSA	01-Jul-05	01-Jul-07
M01040	How can current slaughter, dressing and cleaning procedures in UK pig slaughterhouses be improved to reduce the risk of *Salmonella* contamination of pig meat?	FSA	01-Sep-05	30-Nov-07
845	Development of a risk assessment for *Salmonella* in shell eggs and processed eggs in Ireland	FSPB	01-Oct-04	30-Sep-07

Project Code	Title	Funder	Start Date	End Date
2004108	Interaction of *Salmonella* with antimicrobial peptides	HPA	01-Apr-03	28-Feb-07
2004132	Risk factors for *Campylobacter* and *Salmonella* infection in children aged under one year	HPA	01-Apr-02	31-Dec-08
G0001245	Role of complement and receptors for the Fc Fragment of Immunoglobin G (FcyR) in immunity to *Salmonella*	MRC	14-Jan-02	13-Jan-07
G0501415	Unravelling multiple antibiotic resistance in *Salmonella* enterica	MRC	01-Jul-06	01-Jul-09
G0501449	Quorum Sensing and Host-Pathogen Communication in *Salmonella* Typhi	MRC	01-Feb-07	31-Jan-10
G0600805	The molecular basis, and biological cost, of fluoroquinolone resistance in *Salmonella enterica* serovar Paratyphi A.	MRC	01-Oct-06	30-Sep-09
G9717183	*Salmonella* virulence	MRC	01-Aug-03	31-Jul-08

APPENDIX 2. PROJECTS PREVIOUSLY CAPTURED IN MSFFG REPORTS

Project Number	Location	Project Title	Funder	Start date	End Date
2002029	Risk Report[38]	Genetic characterisation of the mechanism of multiplication of the *Salmonella* Enteritidis in eggs	HPA	01-Oct-01	28-Feb-07
OZ0322	Risk Report	An evaluation of current animal and human *Salmonella* research, harmonisation of diagnostic techniques and introduction of novel research tools	Defra	01-May-05	30-Apr-06
OZ0323	Risk Report	An integrated risk based approach to the control of *Salmonella* in UK pig farms	Defra	01-Apr-05	31-Oct-08
OD2011	Antibiotic Resistance Report[39]	Development of proteomic targeted tests for microbial multiple antibiotic resistant zoonotic food-borne pathogens	Defra	01-Oct-03	31-Mar-07
01-RESR-043	Consumer Practices Report[40]	The development of a risk communication model based upon best practices in the home to facilitate adoption of best practice in disseminating information with a food risk component	FSPB	01-Apr-02	31-Mar-05
B13014	Consumer Practices Report	Determination of the appropriate cooking regimes for the safe roasting/cooking of unstuffed poultry - turkey, chicken, goose and duck	FSA	01-Jun-07	31-Oct-07

[38] Risk Report *UK publicly funded research relating to risk assessment and the microbiological safety of food*. http://www.food.gov.uk/science/research/researchinfo/foodborneillness/microfunders/msffg/msffgriskassessmentreport

[39] Antibiotic resistance report *UK publicly funded research on microbial antibiotic resistance in relation to the safety of food* http://www.msffg.org.uk/reports/antibioticresistance.html

[40] Consumer Practices report *UK publicly funded research relating to food preparation practices and behaviour in relation to the microbiological safety of food* http://www.food.gov.uk/science/research/researchinfo/foodborneillness/microfunders/msffg/msffgjan09