FOOD STANDARDS AGENCY

REPORT OF THE *CAMPYLOBACTER* RESEARCH

PROGRAMME REVIEW

The Kingsley, Thistle Holborn
5th May 2016

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1. Executive Summary

The Food Standards Agency (FSA) has a commitment to review its research programmes on a regular basis. This latest review report is focussed upon the *Campylobacter* research programme, which predominantly covers funded projects that have been undertaken since 2010. The aim of the review was to evaluate the research by taking into account the programme's overall scientific and policy objectives and to consider the possible future directions of research within the programme.

The *Campylobacter* programme review in its entirety was held over three days; the *Campylobacter* research workshop was held over two days at the Chesford Grange Hotel, Kenilworth and the programme review at the Kingsley Thistle, Holborn. The research workshop consisted of oral presentations for the majority of the projects and allowed the attending reviewing panel the opportunity to gather more information about the research and to have discussions with the contractors. The closed review session took place several months later whereby the external reviewers discussed the individual projects in turn and the programme as a whole.

2. Background to the Review

2.1. *Campylobacter* Programme

2.1.1. Rationale for the *Campylobacter* Programme

Initially, *Campylobacter*-related research projects were funded under the old Foodborne Disease Research Programme (B14). The original aims of this programme were:

- to provide robust information on the presence, growth, survival and elimination of micro-organisms throughout the food chain
- to provide the extent, distribution, causes and costs of foodborne disease.

Within this theme, research was commissioned in support of the Agency's strategy to achieve a reduction in the incidence of foodborne disease by 20% over a five-year period. *Campylobacter* was of particular focus in the B14 programme given that it was the major cause of infectious intestinal disease (IID). Work undertaken included the contribution made by the food chain to the problem of *Campylobacter*. Some of the work funded under the previous programme is still ongoing and will be referred to within this report. Further details of the original programme can be found here: https://www.food.gov.uk/science/research/foodborneillness/b14programme

The *Campylobacter* Risk Management programme was implemented as part of the FSA's 2010-2015 Foodborne Disease Strategy. This was as a result of the continued rise in the number of laboratory confirmed cases of foodborne pathogens, in particular *Campylobacter*. The programme encompasses a range of projects targeted at different points across the food chain, from farm to fork.
http://www.food.gov.uk/sites/default/files/multimedia/pdfs/fds2015.pdf

To contribute to this work the Agency also funded research in collaboration with the Biotechnology and Biological Sciences Research Council (BBSRC), Defra, the

Northern Ireland Department for Agriculture and Rural Development and the Scottish Government; the research forms part of a joint strategy entitled: UK Research and Innovation Strategy for *Campylobacter* (UK RISC) in the food chain. http://www.food.gov.uk/sites/default/files/multimedia/pdfs/Campylobacterstrategy.pdf

This helped all departments with a common interest in *Campylobacter* to carry out research in a coordinated and coherent way. As part of these changes, it led to FSA *Campylobacter*-related research being funded under the *Campylobacter* Programme. A summary of the research requirements can be found in **Annex A**.

2.1.2. Objectives of the *Campylobacter* Programme

The scientific aims of the programme are to:

- take forward a coordinated programme of research with other funders to identify and develop effective interventions to control *Campylobacter*

- implement interventions designed to reduce *Campylobacter* levels to our target figure, utilising our engagement with stakeholders and outputs from research to do this

- continue to work to improve public awareness and use of messages about good food hygiene practice at home and in catering establishments to reduce levels of Campylobacteriosis in humans.

2.2. The Reviewing Panel and Attending Officials

The reviewing panel was comprised of eight experts with relevant experience covering food microbiology and epidemiology.

Chairman	
Professor Eric Bolton	Honorary Professor in the Department of Epidemiology and Population Health at the University of Liverpool
External Reviewers	
Professor Tom Humphrey	Swansea University
Mr Alec Kyriakides	Sainsbury's
Professor Noel McCarthy	University of Warwick
Professor Peter McClure	Mondelez International
Professor Sarah O'Brien	University of Liverpool
Professor Norval Strachan	University of Aberdeen
Dr Dan Tucker	Department of Veterinary Medicine – University of Cambridge
FSA Officials	
Mr Adekunle Adeoye	ACMSF Secretariat
Mr Tim Chandler	Foodborne Disease Control

Dr Adam Hardgrave	Foodborne Disease Control
Dr Kevin Hargin	Foodborne Disease Control
Dr Bettina Mavrommatis	Foodborne Disease Control
Mrs Lorna Rowswell	Foodborne Disease Control
Dr Manisha Upadhyay	ACMSF Secretariat
BBSRC Officials	
Dr Sian Rowland	
Adam Staines	

2.3. The Review Process

The overall aim of this review was to assess whether the programme had met its scientific and policy objectives and to make recommendations on the future direction of the FSA's *Campylobacter* programme.

The reviewing panel consisted of individuals whose expertise covered microbiology and epidemiology. Reviewers were assigned an even number of projects to ensure they had the time to carry out their evaluations effectively. Where possible, reviewers were provided with the original research requirement proposal that had led to the work being commissioned, the project proposal/contract and/or scope of work and any interim or final reports (either draft or final depending on stage of project).

The reviewers were also provided with abstract summaries on most of the projects whilst in attendance at the *Campylobacter* research workshop, which was held on 8-9th March 2016 in Kenilworth, Warwickshire. This was also a valuable opportunity to view contractor presentations and to ask questions about the projects that they had been asked to review. A standard evaluation form was provided on which they were requested to submit written comments in advance of the review meeting. The names of the reviewers have been removed to ensure complete anonymity.

The closed review meeting was held on 5th May 2016 in Holborn, London. This was attended by the reviewing panel alongside FSA and BBSRC officials. Approximately 15 minutes were allocated to each project that had been assigned for review. This allowed the reviewing panel to provide their comments and for the chairperson to form an accurate evaluation of the individual projects. It was decided to also provide a rating for science and value for money for each of the projects.

2.4. Outputs from the Programme

Annex B contains abstracts or summaries of the individual projects included in the review, together with a brief summary of the reviewers' comments on each project.

The Scoring System: Each project was awarded two individuals scores during reviewer evaluation discussions. Both 'Science' and 'Value for Money' were marked out of five (5 = excellent, 4 = very good, 3 = good, 2 = average, 1 = poor, 0 = very poor).

The ‘science’ score took into account a number of distinct categories:

- The technicality of the project
- Whether the project achieved its objective/s as set out in the research requirement and/or original scope of work
- Whether the outcomes were relevant/appropriate for the FSA
- Its impact on policy/FSA advice.

The ‘value for money’ score is tied in closely with the science score as this took into account what specific outcomes the FSA obtained against the cost of the project. For example, if the project excelled scientifically and provided the necessary outcomes (both meeting/achieving an FSA research priority and the project’s original aims/objectives) at a low to moderate cost, it would generally be expected that a high (4-5) ‘value for money’ score would be awarded.

N.B. Some projects, in particular those in which the FSA contributed towards grants led by BBSRC or Defra, did not provide official final reports. In FSA contributed BBSRC led projects, under the terms and conditions it was not a requirement for BBSRC to provide a final report. Where appropriate, summary reports were produced by the FSA using key information acquired from interim reports and updates from the individual BBSRC-led projects. When there was an agreement that a lack of documentation would prevent the relevant panel members from carrying out a reliable review of a project, regardless of whether it was FSA-led or not, it was left unscored. This is indicated where relevant.

The costs associated with each project are listed in **Annex C**. Publications arising from the projects under review are listed at **Annex D**. Important examples of FSA policy arising as a result of the outcomes generated through the *Campylobacter* programme, in particular from research, are listed in **Annex E**.

3. The Quality of the Programme and Future Recommendations

The programme has included a range of short and long-term projects which have encapsulated the different sectors of the food chain. The reviewing panel was pleased to see that several projects had been jointly funded with other government departments, notably BBSRC, which was one of the objectives of the 2010-2015 joint action plan.

In some instances, it was felt that the outcomes, or expected outcomes, benefitted one government department over another. It was therefore recommended that any subsequent jointly funded research should by managed by departments that will both have the same desire to see, and use, the project outcomes. If this isn’t equal, the funding percentage should be adjusted accordingly. Overall, the majority of FSA-funded projects appeared to address FSA policy needs.

Given the importance of tackling *Campylobacter*, it was recommended that project teams share data and collaborate on newly identified issues where practically possible and if appropriate. This would greatly benefit research and will help to identify potential solutions in reducing the threat of *Campylobacter*.

There had been some variation in the formatting style of some reports. The Agency does have a standard set of templates for interim and final reports and project officers are reminded to share these with contractors. In some instances, these hadn't been shared and the relevant project teams produced a report against their own templates. The Agency will be ensuring all subsequently final reports are based upon the necessary standard template.

The changing of personnel during the life of the project can have a significant impact on the delivery of any project. This was evident on a number of FSA-funded projects. There was a recommendation for the FSA to consider this when commissioning any new piece of research. Contracts should take this into account and if the current contractor cannot fulfil its duty to carry out work of a continued high quality then it may be appropriate to suspend the project.

A concern was raised about how well the tendering and commissioning of projects had previously been managed. This was because a number of the projects didn't have all of the appropriate documentation, for example the original research requirement. This did make it difficult for some of the reviewers to fully evaluate the projects. FSA officials confirmed that the tendering process had improved and is now being managed effectively. As with all FSA procedures, improvements continue to be made. However, there was a period of time in which a number of the projects were not filed or managed properly. The FSA confirmed that it now uses a new document management system which has helped to improve file storage and the sharing of relevant documents between colleagues.

A small number of projects included in the review were not FSA-led, but were partially funded by the FSA. These projects would have been undertaken as per the joint research call initiative with other government departments, predominantly BBSRC and Defra. The panel expressed concern over a lack of available documentation to evaluate as part of this review, namely official interim reports and final reports. As per the terms and conditions of BBSRC projects, not all final reports are required to be submitted to the FSA. In some cases, the FSA drafted their own version of a summary report based on updates they had received and peer reviewed publications resulting from the research. Future joint programmes with the research councils would benefit from a more comprehensive final report requirement for the FSA. At the very least, it should include updates on individual objectives and impact of the work. There was also a recommendation for FSA Officials to approach the Project Leaders directly to obtain more detailed final reports.

On a related matter, some concerns had been raised previously about why the FSA had partially funded specific projects which, if they were carried out successfully, wouldn't have necessarily produced the outcomes that the FSA desired/generated outputs of importance to the Agency. Given this, the process of allocating funds between the different departments was questioned. The FSA confirmed that this process has been reviewed.

Overall, it was felt that the programme was a partial success due to the outcomes from some of the projects and that the projects broadly covered a number of different areas. However, it should be noted that the projects were of variable quality, which will impact on how well the project outcomes are regarded. There was an indication that more

research could be carried out on the host-pathogen interaction, particularly with the chicken gut and how this affects the microbiota.

Collaboration with industry is important, with consideration necessary on how to do this effectively, especially with outcomes that could potentially result in improving the control of *Campylobacter*. There is sensitivity surrounding Campylobacter within a number of sectors and any policy advice needs to take into better account what impact it will likely have and how to get the right messages across effectively. It would be advantageous for the FSA to regularly seek views and opinions from their social scientists at an early stage.

To further assess individual projects and their overall success to the programme, it was recommended that contractors continue to provide updates on peer reviewed publications to the Agency. In a related matter, it was commented that the Chief Scientist report doesn't tend to cite peer reviewed papers. However, if the first recommendation was carried out, this could be feasible.

It was recommended that any subsequent research should attempt to be as close to a real-life scenario as possible. There should also be consideration for there to be a focus on a smaller number of larger, higher quality projects alongside smaller projects where these address specific FSA priorities given that among our highest scores were projects FS101025 and FS101062, which were small, targeted projects around well-defined FSA priorities. In addition, the involvement of a credible statistician/modeller, depending on the type of project, would work well in partnership. Overall, this may help to improve credibility of individual projects and the outcomes are likely to be easier to rely on.

4. Overall Conclusions

- The majority of the FSA-funded, *Campylobacter*- related projects appeared to address FSA policy needs. They also encapsulated different sections of the food chain.

- The quality of the projects was variable, but most appeared to cover what was felt to be important research. There were a number of good outcomes from some of the completed research. The FSA needs to ensure relevant audiences, for example caterers and food businesses, are able to access the final reports with ease, particularly as some offer important advice and recommendations which could help to reduce the burden of *Campylobacter*-related food poisoning. A consideration should be made to ensure these are prominent on the main website.

- Several of the projects include literature reviews but these are now out of date. There has been considerable output (globally and in the UK) in many areas of *Campylobacter* research and therefore, the reviewing panel recommended that prior to funding future work, more recent literature reviews should be undertaken to inform the FSA funded research.

- Given the sensitivities surrounding *Campylobacter*, it would be of great benefit for the FSA to utilise the expertise of its social scientists to determine how best to handle any potential change in policy advice or recommendations.

- Evidence of jointly funded research had been observed and should be commended; however, all involved departments need to consider carefully the potential outcomes in future research opportunities to determine if they are equally desirable to them. If the level of interest isn't shared evenly, the total funding proportions should be adjusted accordingly. There should also be a consideration to withdraw any funding opportunity if the project outcomes will not be of direct use to that department.

- The terms of any newly commissioned research should include assurances of the project lead remaining in place throughout the life-cycle of the project to ensure the work is carried out effectively. Consideration should be given to introducing project termination clauses which may be of benefit to the FSA should such an eventuality arise.

- The programme would benefit from the inclusion of as many real-life scenario style projects as possible, especially when intervention-focused.

- More attention should be paid to statistical analysis in evaluations of relevant research; the involvement of a credible statistician/modeller during the evaluation process and, where appropriate, to assist throughout the lifecycle of the research project, would be valuable to further support outcomes.

Further information, including final reports, can be found by visiting www.food.gov.uk and typing in the relevant FS number using the search function.

Annex A. Summary of research priorities from the joint strategy entitled: UK Research and Innovation Strategy for *Campylobacter* (UK RISC) in the food chain

Understanding current practice and potential intervention strategies

• High-quality baseline data and regular monitoring of poultry
  - High-quality baseline data
  - Regular measurements of *Campylobacter* levels on poultry

• Comparison of the different on-farm and in-factory practices that affect *Campylobacter* incidence in poultry

• Understanding the effect of water treatment, feed regimes and supplements for poultry

• Studies around potential interventions in poultry transport/slaughter house/factory practices

• Quantitative modelling of interventions
  - On-farm and processing
  - Catering, retail and the home

• Human behaviour:
  - On-farm and in production processes
  - Domestic and commercial preparation and cooking practices

The biology of the host and pathogen

• Predictive modelling of the system
• How the bacterium survives in the food supply chain
• Colonisation in the chicken and the chicken immune response
• Increased understanding of the role of microbiota in the chicken gut
• Development of bacteriophage, bacteriocins and other new anti-microbials
• Development of greater resistance to *Campylobacter* colonisation in chickens
• Underpinning the potential for a cost effective chicken vaccine(s)

Development of novel detection and diagnostic tools, and resources for *Campylobacter* research

• The development of a rapid, on-farm test for *Campylobacter*
• A strain bank to assist in understanding the genetic diversity of the bacterium

Annex B. Review of Individual *Campylobacter* research projects

FS241049 A

Project Title: Development of a rapid, on-farm test for the detection of campylobacters.

Authors: Malcolm Taylor[1], Bob Madden[1], Fiona Young[1], Hywel Ball[2], Mike Hutchison[3]

Institutions: [1]Food Microbiology Branch, Agri-Food and Biosciences Institute Belfast (AFBI)
[2]Bacteriology Branch, AFBI.
[3]Hutchison Scientific Ltd, Broughty Ferry, Dundee, DD5 3EZ

Project number: FS241049 A (M01060)

Start date: January 2011 **End date:** March 2014

Aims of project:

- Phase 1
  - Production of a sample collection, transport and culture protocol
  - Determination of optimal on-farm sample matrix
  - Preliminary evaluation of range of currently available rapid detection methods
- Phase 2
  - Configuration of complete "best approach" methodology into convenient easy-to–use 'farm-proof' format
  - Laboratory and "on-farm" studies (including Model Farms)
- Project extension
  - Extension to include other Integrators / processors
  - Availability of house screening to other FSA projects
  - Introduction to independent growers (NFU)
  - Collaboration with TSB funded isothermal assays.

Abstract:
This project investigated the detection of campylobacters on farms using a range of commercially-available *Campylobacter* detection assays (lateral flow devices, isothermal DNA amplification assays and real-time PCR assays [RT-PCR]).

Initial studies focused on the identification of the optimal broiler house sample type (litter, faeces, boot swab etc) as determined by culture, followed by an investigation of the optimal boot swab type and demonstration of boot swab stability with time and temperature. To identify negative samples for the study, a broiler house screening methodology utilising boot swab sampling and RT-PCR was undertaken. Culture confirmation of the refrigerated samples confirmed *Campylobacter* contamination of these samples.

Each of the *Campylobacter* assays were evaluated against a primary panel of campylobacters (*C. jejuni*, *C. coli* and *C. lari*) and non-*Campylobacter* type cultures. Assay performance against a panel of *Campylobacter* culture-positive and -negative broiler house samples were used together with an evaluation matrix, to identify the optimal assay. The assay with the optimal performance was RT-PCR. The RT-PCR protocol was further validated by confirmation of its specificity against a more extensive secondary panel of type cultures. The protocol repeatability and reproducibility was determined.

In addition the RT-PCR assay was evaluated against all cultured field samples (boot swabs) and rolled out for field evaluations by both large and small processors.

Key Findings:

- Tunika boot swabs were the best for collecting campylobacters from litter.
- Samples were stable at 4oc for at least 24h before culture.
- Samples were stable for at least 4 days at 21oc for RT-PCR, which allowed shipping to the lab without refrigeration.
- RT-PCR was the optimal test method in terms of specificity, sensitivity, reproducibility and cost (approx £12.00 per sample including sampling consumables, postage and laboratory test costs).
- Although not an on-farm assay, the RT-PCR protocol allows results reporting to farmers within 30 h of sample collection in 96% of cases.
- The method now has wide scale adoption from both large processor broiler houses (Industry Biosecurity Group) and smaller independent growers (NFU). Around 300 samples are tested each day.

Outputs:
The provision to industry of an alternative broiler house *Campylobacter* test that is not culture-based, and which is rapid, reliable and easy to implement.

The reviewing panel evaluation

The aim of the project was to develop a low-cost lateral flow test strip for rapid on-farm detection of *Campylobacter* in poultry in accordance with the guidelines described in the FSA/TSB-SBRI Competition. The reviewing panel agreed that this project had appeared to have achieved its primary objectives and presented good value for money overall. However, a concern was raised about the underdevelopment of the on-farm test it had identified as part of its objectives; one could argue that it didn't actually deliver on producing a robust 'on-farm' test. It was also felt that the project team had produced an on-farm test that would be for their commercial benefit going forward. It was further questioned why the use of hens over broiler faeces was used given that their microbiotas would differ. The research team appeared to lack some important knowledge about poultry production. It was recommended that further development of the boot swab testing technique could be valuable. Overall, there still remains the need for development of a low cost, on-farm test.

Science: 3 (out of 5)
Value for money: 3 (out of 5)

FS241049 B

Project Title: Development of a rapid on-farm Test for the Detection of *Campylobacter* in Poultry

Authors: David Reddick[1], Jay Modha[2]

Institutions: [1]Moredun Scientific
[2]Modha Biomedical

Project number: FS241049 B (M01061)

Start date: March 2011 **End date:** March 2014

Aims of project:

- **Objective 1**: Development of *Campylobacter* immunoassay and porting to lateral flow strip test platform
- **Objective 2**: Production and evaluation of prototype lateral flow test strips
- **Objective 3**: Field testing of *Campylobacter* detection test kit
- **Objective 4:** Optimisation of new prototype lateral flow strip

Abstract
The aim of the project was to develop a low-cost lateral flow test strip for rapid on-farm detection of *Campylobacter* in poultry in accordance with the guidelines described in the FSA/TSB-SBRI Competition. The purpose of the test was to enable poultry farmers to monitor the *Campylobacter* status of their flocks in order to assess the success/failure of biosecurity procedures.

Optimisation assays identified a number of different antibodies which gave a strong signal using a crude direct ELISA and further optimisation identified the most appropriate filter pads, membrane and conjugates. With this basic assay, sample testing using *Campylobacter* spiked poultry faeces was carried out to confirm that the assay could identify *Campylobacter* in the faecal matrix and to identify any problems with the assay design. Testing did confirm that the assay would detect *Campylobacter* in faeces, however the level of detection (LOD) of the assay was reduced by 1.5 log, from $\log_{10}$ 6.0 to $\log_{10}$ 7.5 cfu/mL, when testing was performed using spiked faecal samples in comparison to *C. jejuni* prepared in buffer alone, due to blocking of the filter pad with faecal material.

Using the optimised material, a batch of prototype strips was prepared in two formats, dipsticks and cartridges, for use in lab testing using *Campylobacter*-negative faecal samples spiked with various concentrations of *C. jejuni* and *C. coli*.

The laboratory testing demonstrated that the experimental test strip could detect *C. jejuni* and *C. coli* if faecal samples comprised ≥3x10^7 cfu/g with a CV of 20%.

Further lab testing demonstrated that chicken faeces caused assay interference with a loss of sensitivity of at least 1 log cfu/mL compared to *Campylobacter* in buffer alone. Further work was necessary to optimise the test strip and this was done by replacing the original glass fibre filter with a slightly thicker cellulose-based filter and increasing the run time to 15 minutes.

Following optimisation, the assay was rapid (result in <15 minutes), was easy to perform on-site and detected *C. jejuni* and *C. coli* in faeces from chickens with ≥ $3x10^7$ cfu/g or mL faeces. The test strip required an inoculum of ~10^7 total cfu in order to produce a *strong* visible signal.

Following review of the strip performance it was observed that using a portable reader to quantify (and digitalise) the signal reported by the strip, increased strip sensitivity, adding an extra 1 log cfu/mL to its performance. This increased the LOD down to ~10^6 cfu/mL faeces, which was equivalent to 10^5 cfu total in the 100µL inoculum that is applied to the test strip.

Field testing demonstrated that although both types of test strips operated correctly, all the test strips reported negative results with faecal samples and with boot swabs in comparison to conventional microbiological methods. This was determined to be due to a lower than expected *Campylobacter* colonisation level in the tested flocks which was lower than the LOD of the test ($3x10^7$ cfu/mL (or $\log_{10}$ 7.5). This was supported by conventional microbiology data which showed that the average number of *Campylobacter* in the samples tested ranged from log 4.6 – 5.7 cfu/mL (i.e., $4x10^4$ to $5x10^5$ cfu/mL) which are all below $3x10^7$ cfu/mL, the LOD of the test strip.

Further optimisation of the test strip was required to increase the sensitivity by 2.5 – 3 logs in order to detect a signal from low to moderately contaminated samples. Modifications were suggested to increase sensitivity by three logs in total including using a biotin-streptavidin signal amplification system and colloidal gold as a label and optimising buffer strength.

A total of 42 experiments were conducted aimed at optimising the sensitivity of the test strip. This resulted in new versions of test strip being developed using latex and colloidal gold nanoparticles as the label, which both proved to increase sensitivity.

The LOD of the latex labelled strip was found to be 1.5 log better than its predecessor thus the LOD improved from log 6.6 to log 5, and experiments with the gold detection conjugate suggested that an LOD of between log 3 and log 4 cfu/mL was possible although this work could not be completed.

The objective of the project was to develop an easy-to-use and low-cost test for rapid on-farm detection of *Campylobacter* in poultry. With the optimised assay a detection level of log 5 cfu/mL was produced with possible further improvement to log 4 cfu/mL.

This level of sensitivity would allow detection of contaminated poultry flocks colonised with low, medium or high levels of *Campylobacter* spp which would meet industry demand.

Some unique features of the *Campylobacter* test strip developed are described below:-

- **Rapid** on-farm test for *Campylobacter*; results within 15 minutes. A poultry house can be [flock] tested within 30 minutes of sampling
- **Easy-to-use**; no complex sample preparation
- **Specific** for *Campylobacter*; detects *C. jejuni* and *C. coli*
- **Sensitive**: LOD of ~log 4-5 cfu/mL
- **Can be used for any sample type**; boot swabs, faeces and caecal droppings
- **Low-cost**; a poultry house could be tested for around £15 (using two pooled samples per house)

Available in two formats; dipstick or cartridge device (dipstick not suitable for LFD reader)

The reviewing panel evaluation

The sensitivity issues observed in the outcomes could and should have been predicted by the project team. Despite additional funding used for extending the work, the project team continued to experience some problems. This also appeared to be a failure of not referring to the interim review, which would have helped to ensure the project progressed in the appropriate manner. The lateral flow test developed failed to indicate when a flock became positive. There should have been a thorough examination of lateral flow devices and how farmers can use them in an on-farm setting. The credentials of the project team were also questioned, given that it was felt that they did not have the necessary experience of working in poultry production. It was further questioned why both FS241049 A and FS241049 B were funded separately, and perhaps one project team could have carried out a more streamlined piece of research. Overall, this project didn't fully meet its objectives and could be considered to be less of a success than FS241049 A. There is potential for further application but it would need some considerable further work; however there is no guarantee of success.

Science: 2 (out of 5)
Value for money: 2 (out of 5)

FS101114

Project Title: Epidemiological analysis of *Campylobacter* data generated in an industry biosecurity study

Authors: M. Georgiev, W. Beauvais[1], J. Guitian[1]

Institutions: [1]The Royal Veterinary College

Project number: FS101114

Start date: January 2014 **End date:** April 2015

Aims of project:

- To analyse information collected by the industry on *Campylobacter* colonisation of poultry batches originating from farms with enhanced biosecurity and from control farms with standard biosecurity.

Abstract

The aim of this study was to analyse information collected by the industry on *Campylobacter* colonisation of poultry batches originating from farms with enhanced biosecurity and from control farms with standard biosecurity. The study supports the activities of the FSA and the Joint Working Group on *Campylobacter* (JWG) aimed at reducing levels of *Campylobacter* spp. colonisation in poultry at farm level in the UK.

The hypothesis that enhanced farm biosecurity contributes to a decrease in the risk of *Campylobacter* colonisation at high levels was tested. Furthermore, the contribution of partial depopulation, empty days between flocks in the sheds, type of hybrid and season to the probability of batch colonisation with *Campylobacter* at high levels was quantified. Finally, the data were used to compare the effect on our results of assessing batch status by means of pooled caecal samples versus pooled neck skin samples. The analysis includes 1,749 batches originating from 16 'model' farms with elevated biosecurity and 565 batches grown in farms with standard biosecurity between September 2011 and August 2013.

For each batch, data were collected on selected factors/characteristics and the levels of *Campylobacter* spp. were measured in pooled caecal and pooled neck skin samples. All samples were tested according to ISO10272-2 2006. The results of microbial testing of caecal samples were used to classify batches initially according to the same threshold targets agreed by the JWG and applied by FSA, for monitoring at neck skin samples, in three bands: Low (<100cfu/g), Medium (100 to ≤1,000cfu/g) and High (>1,000cfu/g). However, because bacterial counts can be expected to be lower in neck skin samples than caecal samples, the analyses were conducted using an increased threshold level (5.09 $\log_{10}$ ≈ 123,000 cfu/g) for the classification of batches as positive (highly colonised).

Following the identification of two suitable control groups of farms with standard biosecurity from datasets provided by the industry, statistical analyses were carried out for one factor at a time (univariate); and adjusting for confounding factors (multivariate), to assess the relationship between selected 'on farm' factors and the probability that the batch was highly colonised. The findings support the following conclusions:

- A substantial risk of *Campylobacter* spp. infection is present on farm (i.e. in the beginning of the food chain), as a large proportion of all the poultry batches included in this study (58.6%) were colonised in caeca at high levels.
- The risk of batch colonisation exhibits seasonality, with a peak in summer when almost 90% of the studied batches were highly colonised.

- Enhancement of biosecurity in commercial poultry farms can contribute to the reduction of batch colonisation at thinning to 60.0% (123,000 cfu/g threshold) of the risk in control farms. Following thinning, the potential effect of increased biosecurity becomes much less apparent.

In addition to the season, husbandry factors such as the practice of partial depopulation (thinning), hybrid type and empty period between flocks in a shed were also associated with the probability of batch colonisation at high levels >123,000cfu/g. In farms with enhanced biosecurity, batches in which thinning had been previously carried out were significantly more likely to be colonised at >123,000 cfu/g in caeca than batches in which thinning had not been practised (66.6% vs. 48.4%). An empty period of less than one week between the flocks decreased the risk of colonisation at >123,000 cfu/g in caeca to the level of 51.2% compared with the risk 54.4% if empty period was 1-2 weeks. A prolonged empty period of >three weeks between the flocks increased the risk of high colonisation to 72.9%.

The reviewing panel evaluation

The reviewing panel agreed that this project was incredibly good value for money and produced outputs of high quality which enabled robust evidence to be gathered. It fully met aims and objectives. It was a single tender project that followed on from an earlier piece of work by RVC. The work carried out helped to support the identification of thinning as being a major risk factor in leading to the contamination of chicken. Based on the outcomes, it also highlighted that the duration of emptying time was a significant factor; longer emptying times helped to reduce the amount of *Campylobacter* within the broiler sheds. Further understanding/dissection of these biosecurity factors was recommended. The reviewing panel stressed the importance of preserving the biosecurity at all times and for industry to take note of these results. It was felt that more social science input was needed in order to change the mind-set. There was also a recommendation for further work to help support these findings. The final remarks were that the report was very well written and the project team impressed in delivering this piece of work. The outcomes helped to further support what was already known.

Science: 3 (out of 5)
Value for money: 5 (out of 5)

FS101123

Project title: A new on-farm *Campylobacter* testing provision covering the independent broiler farming sector across the United Kingdom.

Authors: M. L. Hutchison[1], M. J. Taylor[2], and G. Ford[3]

Institutions: [1]Hutchison Scientific Ltd, Broughty Ferry, Dundee DD5 3EZ
[2]AgriFood Biosciences Institute, Belfast, Northern Ireland BT9 5PX
[3]National Farmers' Union, Stoneleigh Park, Kenilworth CV8 2LG

Project number: FS101123

Start date: October 2014 **End date:** January 2016

Aims of project:

- Identification of risk factors for the colonisation of chicken broilers on non-integrated, independent farms.
- To raise awareness of the issues caused by campylobacters amongst independent growers and the importance of keeping birds un-colonised.

Abstract

Previous work funded by the FSA as study FS241051 identified that 2/9 of the variance in *Campylobacter* contamination observed on chilled chickens was explained by slaughterhouse processing factors. The remaining 7/9 of the variance stemmed from on-farm factors. Consequently, this study was concerned with the identification of on-farm factors that influenced bird colonisation.

Farmers were asked to sample house litter using boot swabs immediately before the birds were caught and sent for slaughter. Matched with the litter sample was information provided by questionnaires with set responses. The questionnaires included farm details and descriptions of the broiler sheds, both of which were likely to change infrequently and so were answered only once. In addition, a questionnaire that captured information likely to change between batches of farmed birds was returned for every litter sample submitted for testing.

Litter testing was by quantitative PCR, which removed any requirement for sample refrigeration during transit.

Questionnaire data and test results were held in a relational database and linked using a primary key consisting of a farm identifier, shed number and sample collection date. Information from 1,780 flocks, with birds aged from 26 to 50 days at the time of testing was used to construct a hierarchical linear model to properly account for the correlation structure within the collected information. In the model, a three-level hierarchy was specified as: livestock batch, house identifier and farm identifier. The model calculated the terms exerting significant influence to the $\log_{10}$ *Campylobacter* numbers.

The model indicated that:

- There was also an overall protective effect for some bird genders. Female birds had a geometric mean that was 1.107 $\log_{10}$ cfu/g lower compared with male gender birds ($p < 0.001$). It is common in the UK for the lighter female birds to be cleared from houses first, with the males allowed to grow on to a greater weight. The underlying reason for that practice is because cocks have the capacity to grow to a heavier weight and achieve a better feed conversion ratio compared with pullets.

- If prebiotics were fed to birds, the $\log_{10}$ count was increased by 1.400 ($p < 0.001$). The reasons for the finding were not immediately obvious.

- Farm category also exerted an influence on $\log_{10}$ *Campylobacter* numbers. More specifically, compared with independent farms supplying independent processors, independent farms supplying integrated processors and not testing before the commencement of the study had lower counts in their litter by around 1.091 $\log_{10}$ cfu/g ($p = 0.001$). In contrast to integrated processors, independent slaughterhouses tended to favour heavier weight birds because a higher percentage of carcasses tended to be for breast fillet rather than sold whole. One likely further contributing factor was final clearance male birds destined for an independent slaughterhouse may experience catching in their house as many as six times during their life.

There was also an overall effect of the type of house construction on *Campylobacter* numbers with those broiler houses constructed from metal frames having 0.462 $\log_{10}$ counts greater than those with wooden frames ($p < 0.001$). Steel-framed houses are generally stronger than an equivalent timber frame. Consequently, steel frames can be used to construct larger sheds than timber framed ones. Larger sheds can hold larger numbers of birds, and so the protective effect of wood framing may simply be a proxy of numbers of birds placed and the number of depopulations, stress events and exposure to catchers required to clear the shed. Finally, independent processors have a lower processing capacity compared with integrated processors, which lowers the numbers of birds than can be harvested during depopulation.

The reviewing panel evaluation

The reviewing panel had mixed opinions about this project. It was agreed that it did achieve its main objective of reaching out and engaging with independent farmers. The reviewing panel commented that, at the very least, there was a need to continue to address that point. As a consequence this would likely increase their understanding of *Campylobacter*, which would be a long-term benefit in helping them to better manage the status of their flocks. Once farmers became familiarised with the sampling procedure, they would be able to continue this into the future, outside the reporting system of this project.

There were several criticisms about the sampling method used within the study. The measuring of *Campylobacter* levels in litter was not considered to be a wholly accurate representation of the scale of *Campylobacter* colonisation within the flock. It was merely better suited for providing presence/absence status, which it did when reporting the results back to farmers. The expectations were perhaps too high when interpreting the results in a quantitative manner. The lack of completed responses resulted in a significant amount of information not being included in the analysis; this should have been predicted and provision should have been made to follow up on this rapidly where appropriate.

In terms of all of the objectives, it was not possible to determine whether they were all achieved. It was observed that not all of the possible contamination factors were taken into consideration, which significantly impacted on the credibility of this work. In relation to the reporting, it was concluded that the outcomes identified did not offer any new or surprising information. It was also difficult to challenge any of the conclusions presented. The results could be explored further to consider their significance, in particular; sex of the bird and feed withdrawal on colonisation.

It was felt that the project was essentially a repeat of work which had been carried out before in a different way. Overall, the project from a social science aspect was very good, however it was weak in terms of the core scientific approach used.

Science: 1 (out of 5)
Value for money: 2 (out of 5)

FS241018

Project title: Continued development of the Agency's slaughterhouse hygiene tool, including extension for use on-farm

Authors: C. L. Watkins[1], D. Harrison[2], M. A. Tchórzewska[2], V. A. Allen[2] and M. L. Hutchison[1,2]

Institutions: [1]Hutchison Scientific Ltd, Broughty Ferry, Dundee DD5 3EZ
[2]School of Veterinary Sciences, University of Bristol, Langford, Bristol BS40 5DU

Project number: FS241018

Start date: 1st April 2012 **End date:** 30th March 2015

Aims of project:

- Update the peer-reviewed scientific evidence that is used to support the FSA slaughterhouse hygiene assessment tools.
- Update the support pages relating to poultry carcass washing as preparation for possible future policy changes at a European level authorising the use of wash water aids such as lactic acid.
- Identify new processing factors that could be used as the basis of new assessment questions.
- Modify the FSA chicken process hygiene tool so it could be compared with an analogous tool created by the University of Ghent (FSA project FS121014 E).

Abstract

The slaughterhouse assessment tools were originally created by FSA project MO1020. Amongst other things, the original study was concerned with ways that traditional organoleptic meat inspection could be modernised to include provision for not visible hazards such as microorganisms. The original study ran from 2005, which was the same time that statutory testing of carcasses for HACCP-style process monitoring was introduced in the European Union. Assessment tools were created originally for slaughterhouses processing cattle, sheep, pig and chicken broiler carcasses.

The basic idea underlying the FSA assessment tools was that comprehensive reviews of the literature relating to each stage of the processing of each species were undertaken. Processing hazards and beneficial practices, in terms of changes to general microbiological indicators or potential human pathogens were identified. A series of questions were phrased around each identified risk or protective factor. The responses to each question were assigned a score, which was set partly by the number of publications that had identified each particular factor, a lack of conflicting reports and the expert opinion of a panel of 45 research scientists and industry representatives. The output from a hygiene assessment was a summary numerical score, calculated from weighting the scores from the individual processing stages. Stage weightings were related to the potential contribution made to the overall process hygiene (e.g. stages involving heat were heavily weighted) and were also set by expert opinion.

The suitability of slaughterhouses for process hygiene assessment using the tools was determined by a set of 97 pre-requisite questions. Many of the assessment questions required measurements to be taken (e.g. scald tank water temperature); the prerequisite questions related to the integrity of the plant infrastructure, vermin and insect control and the calibration

of instruments used to make the measurements required to answer some assessment questions.

In addition to processing stage scores, related hygiene indicators such as statutory microbiological test results were included as part of the assessment. A SOAP (simple object application protocol; a way for computers to exchange XML data) was installed between the database holding the UK slaughterhouse industry statutory test results and the web server hosting the hygiene assessment) so that test results were required to be entered by slaughterhouse operators only once and could be used for assessment purposes.

Processors were provided with easy-to-use reports that summarised the results of their most recent hygiene assessment, their historical assessments and allowed comparison with other plants (within a parent company) and with the (anonymised) national dataset. The Universities of Bristol, Ghent and Utrecht were provided with 'spider webs' (radar charts) that were broadly comparable to those generated by the Ghent tool.

The reviewing panel evaluation

The reviewing panel agreed that at some stage the tool had been particularly useful for industry to record their *Campylobacter* data on. It also allowed industry members to compare their results to the national average. For that reason, it was considered to be worthwhile. There wasn't any evidence to suggest that the tool had been adapted to be used on-farm, which was the very purpose of this continued development project. Very little documentation was available for which the reviewing panel could refer to in order to make an effective evaluation. The scores below took this into consideration. At time of review, the website had been taken down, however it was recently placed back onto the servers.

Science: 2 (out of 5)
Value for money: 1 (out of 5)

FS121014 A

Project Title: Efficacy and Practicality of Rapid Surface Cooling, Electrolysed Water, Ultra-Violet Radiation, Steam, and Hot Water for *Campylobacter* Reduction

Authors: Dean Burfoot[1], Liz Mulvey[1], Emma Foy[1], Rob Turner[1], Keith Jewell[1], Viv Allen[2], Dawn Harrison[2], Victoria Morris[2]

Institutions: [1]Campden BRI
[2]University of Bristol

Project number: FS121014 A (M01058)

Start date: 01/02/2011 **End date:** 31/12/2013

Aims of project:

- To gather information from equipment providers, the poultry industry, and scientific literature on the efficacy of selected currently available interventions
- To develop an agreed protocol for microbiological sampling
- To carry out preliminary and factory tests of the interventions

Abstract

Data on the efficacy of selected interventions to reduce *Campylobacter* on whole chicken carcasses was obtained from many sources and presented in preliminary and final reports. The interventions included rapid surface chilling, electrolysed water, ultra-violet radiation, chlorine dioxide, steam, and hot water. Trials of the interventions were carried out at poultry processing sites using freshly eviscerated birds.

Trials were carried out immersing breast skin samples in liquid nitrogen or spraying whole carcasses with liquid nitrogen in either a batch chamber or an in-line tunnel. Although immersion of carcasses in liquid nitrogen is not a practical option, the trials showed a 1.3 log reduction in numbers of *Campylobacter* when using immersion for 20s. Spraying of liquid nitrogen for 20s in the chamber achieved a 1 log or greater reduction in *Campylobacter* on whole carcasses. When spraying in the tunnel, a 1.1-log or greater reduction in *Campylobacter* was achieved with a 40s treatment.

Trials were carried out in which whole carcasses were sprayed with electrolysed solutions of sodium chloride or sodium carbonate. Out of 7 conditions tested, only one trial showed a statistically significant effect of the electrolysed water on *Campylobacter* numbers and that was only a 0.3 log reduction.

Previous research had indicated that ultra-violet radiation could produce large reductions in the numbers of *Campylobacter* in water (2 log reduction using 3.4 mJ/cm^2) and inoculated on agar (6 log reduction). Lower reductions had been found with *Campylobacter* inoculated on to breast skin (0.8 or less at 0.19 mJ/cm^2). Tests at 0.24 mJ/cm^2 found no significant evidence of a reduction in *Campylobacter* on breast skin. This result was in agreement with extrapolation of the results from the tests with *Campylobacter* in water.

A D-value is the time required to achieve a 1 log reduction in the number of microorganisms. The D-value for *Campylobacter* at 55˚C on chicken meat is around 1 minute (longer times reported in other substrates) and around 4s at 75˚C. However, the proteins in chicken meat and skin begin to denature (cook) between about 50˚C and 65˚C. Earlier trials by other groups, and trials in this project, using heating times of 10s or more, found changes to the appearance

of whole birds after treatments with hot water or steam. This information suggests that thermal treatments would not be useful as *Campylobacter* interventions on poultry. Nonetheless, very short time treatments using Sonosteam (steam and ultrasound) or hot water are being used/tested by the poultry industry. Extrapolation of results from one paper published in 1995 suggests that the appearance of chicken meat is not affected by treatments of 1.8 s at 90˚C or 2.7s at 80˚C and this could be the reason why the thermal treatments currently being tested might be acceptable. This would particularly be the case if some slight change to appearance is accepted to achieve good *Campylobacter* reductions.

The reviewing panel evaluation

The project addressed the requirement M010R0017 on slaughterhouse interventions to reduce *Campylobacter*. The project achieved most of its objectives as set out in the original agreement. The reviewers noted that it didn't carry out its plan of using water treatment as a potential option. There was also a problem with the chlorine dioxide trial, which reviewers felt could have been repeated to see if the issues could be resolved. Overall, it was concluded that the treatments assessed within this part of the project weren't very successful at removing *Campylobacter*. The reviewing panel felt that this may have been different had the investigators attempted different ranges, in particular, making reference to temperature within the water treatments used. Some combinations involving different interventions were also omitted. The final report described a good synthesis of existing knowledge from the literature and industry with experimental studies to give evidence for each treatment considered. If further work is to be considered, then this could be in one area which was not previously covered, to assess the potential synergistic effect of combining several interventions and their effectiveness.

Science: 3 (out of 5)
Value for money: 3 (out of 5)

FS121014 B

Project Title: Efficacy and Practicality of Lactic Acid Solutions, Ozonated Water, and Cold Plasma for *Campylobacter* Reduction

Authors: Dean Burfoot[1], Liz Mulvey[1], Keith Jewell[1], Emma Foy[1], Rob Turner[1], Emma Maguire[1], Viv Allen[2], Mike Hutchison[2], Victoria Morris[2], Dawn Harrison[2]

Institutions: [1]Campden BRI,
[2]University of Bristol

Project number: FS121014 B (M01059)

Start date: 1st February 2012 **End date:** 31st March 2013

Aims of project:

- To gather information from equipment providers, the poultry industry, and scientific literature on the efficacy of selected interventions not currently "allowed" in the EU
- To carry out preliminary and factory tests of the interventions

Abstract

Data on the efficacy of selected interventions to reduce *Campylobacter* on whole chicken carcasses was obtained from many sources and presented in preliminary and final reports. The interventions included lactic acid solutions, ozonated water, ozonated carbon dioxide pellets, and cold plasma

Trials to test the efficacy of spraying lactic acid were carried out using an in-line tunnel, a sprayer and an electrostatic sprayer. Based on previous work, a pH of 4.0 was used. Treatment times were between 5 and 21 s, acid concentrations were between 4 and 8%, and application rates were from 9 to 145 g of acid solution per kg of chicken. Most treatments produced a reduction in the number of *Campylobacter* of less than 0.5 log. However, two treatments produced higher reductions: a 21s treatment of 8% acid produced a 1.9 log reduction on breast skin but appearance was affected. A 7s treatment of 4% acid produced a 0.8 log reduction of *Campylobacter* on back/neck skin.

Trials with the spraying of ozonated water showed no significant reduction in numbers of *Campylobacter* when compared to the use of water alone. A trial using cold plasma applied in a small chamber (0.26 m x 0.26m x 0.17m) and an ozone concentration of 270 ppm had no effect on the numbers of *Campylobacter* on chicken breast skin.

Data was supplied by a gas supplier on the effect of ozonated carbon dioxide pellets: CO_2 pellets incorporating O_3 and given the trade name Blue Ice. In trials, dry ice consisting of CO_2 pellets produced a 2.1 log reduction in *Campylobacter* that had been inoculated onto non-food surfaces. Blue ice produced a 3.9 log reduction in *Campylobacter*. In trials with poultry, the numbers of *Campylobacter* were reduced by 0.5 log, 0.8 log, and 1.3 log respectively when applying wet ice, dry ice, or blue ice to the surface of the poultry. The gas supplier no longer produces Blue Ice.

Some funding under this project was also allocated to extensive trials with rapid surface cooling. That work is described under FS121014.

The reviewing panel evaluation
It was concluded that the project was quite expensive; questions were raised about the allocation of money to specific interventions, in particular a higher proportion of money was used to investigate steam treatment. The assessment reports of the individual interventions investigated were considered to be quite brief. The project assessed the effects of lactic acid, ozonated water and cold plasma. The effects of ozonated CO_2 pellets was not undertaken as the commercial supplier of this technology decided not to produce them. It would appear that the majority of the objectives have been met. Overall, the literature review was considered quite poor. Although the outcomes were clearly identified, all reviewers were in agreement that it did not provide an overly detailed description/critique of them, which would have complemented the overall findings of the research. There were also concerns with the statistical power. It was noted that the interventions investigated were not permitted in the UK and not in commercial use. Some praise was given to both this project and the other closely linked project (FS121014 A) for at least trying to carry out some aspects of the work within a 'real-life' setting.

Science: 3 (out of 5)
Value for money: 3 (out of 5)

FS241051 A

Project title: The monitoring of *Campylobacter* numbers in UK poultry carcasses and collection of information from primary production and processing for risk factor identification.

Authors: M. L. Hutchison[1,2], C. L. Watkins[1], D. Harrison[2], S. Gonzales-Bodi[2], J. E. L. Corry[2], T. Knowles[2], V. A. Allen[2], R. H. Madden[3], P. Scates[3], L. Moran[3] and M. A. Tchórzewska[2],

Institutions: [1]Hutchison Scientific Ltd, Broughty Ferry, Dundee DD5 3EZ
[2]School of Veterinary Sciences, University of Bristol, Langford, Bristol BS40 5DU
[3]AgriFood Biosciences Institute, Belfast, Northern Ireland BT9 5PX

Project number: FS241051 A (M01056)

Start date: 1st July 2011 **End date:** 30th March 2016

Aims of project:

- Collection of UK poultry processing industry *Campylobacter* test results
- Undertake an assessment of the implications of change of sample type from chicken carcass neck skin to neck extension (breast) skin
- The on-going collection of plant characteristics, operations data, equipment and layouts in 20 UK processing plants
- The creation of systems to summarise and report captured information (with appropriate identity safeguards) to make it available to partner projects, industry representatives and Agency staff
- Undertake assessments of the quality of *Campylobacter* testing results by proficiency testing
- The provision of assistance to atypical under-performing testing laboratories
- Multivariate statistical analyses of collected information to determine what processing practices influence carcass contamination by campylobacters.

Abstract

The standard test for industry-funded testing for campylobacters is five samples each consisting of three pooled neck skins collected after carcass chilling. The study assessed the implications of changing the test sample from neck to breast skin. The numbers of campylobacters in breast skin were determined to be significantly higher (t-test, $P<0.05$) on neck skin compared with breast skin. There was no correlation between neck and breast skin *Campylobacter* numbers.

Between July 2011 and June 2014, over 25,000 *Campylobacter* test results were collected from 22 poultry processing plants and stored in a relational database. The data revealed that geometric mean numbers of campylobacters on chicken carcass neck skins increased over the duration of the study.

The quality of the industry-provided *Campylobacter* test results was assessed by eight rounds of proficiency testing. Each round had between 16 and 23 participating laboratories. A range of proficiencies was observed. Commonly-encountered issues included false-positive and -negative results reporting and an inability by some laboratories to consistently convert raw

plate counts into reportable numbers of campylobacters. Data from poorly-performing labs was excluded from the results database. Six staff members from underperforming contract labs were retrained by the Bristol laboratory. Supervisors in 20 laboratories made use of exercise problems (and fully-explained answers) that were prepared to teach the conversion of raw counts to reportable numbers. Four processing companies changed their testing laboratory as a consequence of poor performance over several rounds of proficiency testing.

Online, validated data entry forms protected by secure login were created using the .NET programming framework to capture information describing the growing of broilers on farm and conditions in the plant during processing. The processing conditions data was more detailed than the farm information. Reports that summarised the collected information were created and made available to project partners and industry. The farm and plant information was used to construct a multi-level, Poisson model that attempted to predict the numbers of campylobacters on neck skins. The model revealed that there were nine units of variation that determined *Campylobacter* load on carcasses. On farm growing practices accounted for 7/9 of the observed variation, with processing plant activities accounting for the remainder.

During processing, the timely repair of a failed inside-outside washer (P=0.05), the temperature of the carcass after chilling (P=0.032), meeting the pluck effectiveness target (P=0.0082), meeting the chiller cleaning frequency target (P=0.018) and the season of sample collection [cos(day number) P=0.018; sin(day number) P=0.00052)] were identified as factors that influenced *Campylobacter* numbers on neck skins. A separate presence-absence (binomial) model confirmed the importance of on-farm factors compared with processing conditions for the presence of campylobacters on carcasses. A key recommendation of the study was that more information relating to bird growing was required to identify on-farm risk factors for bird colonisation by campylobacters.

The reviewing panel evaluation

This project acquired a considerable amount of data and was praised for achieving that. Collection of *Campylobacter* test results from six UK processors, which included the comparison of neck and breast skin swabs, was achieved. The standardisation of laboratories was also considered excellent. The multivariate analysis revealed the 7/9 of variance in *Campylobacter* load was explained by farm practices. A number of processing steps were associated with the remaining 2/9 of variance. The reviewers did express difficulties in reviewing this project effectively given the limited documentation. It was also not possible to determine if it had met all of its original objectives, despite the work being extended on several occasions, and to form an opinion of the quality of work delivered. However, it was possible to gauge that a substantial amount of work had been carried out and that there was significant overlap with part B (APHA-led) and the Campytools project (FS121014 E). Given the latter, coupled with the short comings of a final report, the reviewing panel were of the opinion that it didn't represent good value for money.

Science: 2 (out of 5)
Value for money: 1 (out of 5)

FS241051 B

Project Title: Monitoring programme for *Campylobacter* in broiler flocks and broiler carcases in the UK

Authors: Ana Vidal (Project leader)[1]

Institutions: [1]Animal and Plant Health Agency (APHA)

Project number: FS241051 B

Start date: 1st November 2011 **End date:** 30th June 2016

Aims of project:

- Monitoring of *Campylobacter* in broiler slaughter batches and broiler carcasses at national level (Objective 1)
- Validation of laboratory techniques for *Campylobacter* speciation (Objective 2)
- Validation and comparative study of two sampling methodologies for carcase enumeration and investigation of certain parameters of the ISO direct plating enumeration and detection method (Objective 3)
- Collection and analysis of primary production and abattoir processing data (Objective 4)
- Molecular typing (Objective 5)

Abstract

In December 2010 the Food Standards Agency announced a joint government and industry target to reduce the percentage of chickens produced in UK poultry slaughterhouses that have the highest level of contamination, i.e. those with more than 1,000 colony forming units per gram of carcase neck skin samples, from a baseline of 27% in 2008 to 10% by April 2015.

As part of this project, the APHA has conducted a *Campylobacter* monitoring programme of slaughter batches and carcasses together with data collection, speciation and typing of isolates during 2012-2015 to allow the levels of *Campylobacter* contamination to be measured and any significant change in load of *Campylobacter* to be detected over time, in response to the implementation of specific intervention practices at different points along the production chain. This monitoring programme has provided baseline data to feed into risk assessment models and will be used to measure on-going progress on the effectiveness of the FSA Risk Management Programme towards achieving the reduction target. The survey design and sampling protocols were based on the EU technical specifications for EC decision 2007/516. One carcase per slaughter batch was sampled after chilling and before further processing. The samples were then transported to the laboratory for detection, quantification and speciation of *Campylobacter* spp. based on the methods described in ISO 10272:2006.

Furthermore, it is not known how representative a single carcass sample might be in the survey population in a UK context, so the interpretation and conclusions that can be drawn from the studies are currently limited. With this in mind we carried out intensive sampling of a subset of batches aimed at providing additional data on variability that can be used to assist with the interpretation of the monitoring survey. This will contribute to the knowledge of the variability of *Campylobacter* on carcasses, both at quantitative and qualitative levels in a representative and randomly selected population.

Finally, an assessment of the potential for the Matrix Assisted Laser Desorption Ionisation (MALDI) to provide a faster identification and speciation of *Campylobacter* colonies for the enumeration method compared to the biochemical method was carried out.

Main findings:

- The prevalence of highly contaminated carcases decreased from 30.2% in Year 1 (March 2012-February 2013) to 24% in Year 4 (March 2015-December 2015).
- Statistical analysis of the first two years of the monitoring programme showed that increasing bird age, abattoir, line speed, presence of skin lesions, presence of processing damage, mortality at 14 days and increasing proportion of dead-on-arrivals were all included as significant independent risk factors for the most heavily *Campylobacter*-contaminated carcases. These findings provide a robust estimate of the percentage of chickens that are highly contaminated in the UK and the risks associated with *Campylobacter* colonisation in the UK broiler population.
- The number of *Campylobacter* on the carcase samples was correlated with the concentration of contamination in the caeca (most of the highly contaminated carcases were obtained from batches with high loads of *Campylobacter* in the caeca ($>10^6$ cfu/g) and the strength of this association varied within and between abattoirs, suggesting differences in hygiene control.
- *Campylobacter* counts in carcase samples varied within a batch from a minimum of 1.4 to a maximum of 3.6 log10 cfu/g (EUM) and from a minimum of 0.87 to a maximum of 3.12 log10 cfu/g (IHM).
- The sensitivity of detecting a batch with highly contaminated carcases increases as the number of samples tested increases and the difference between a pool of three neck skins and one single neck skin method decreases as the number of samples tested increases.
- The within and between batch variability of *Campylobacter* counts in carcase samples and the impact of different sampling and testing strategies should be taken into account for a correct estimate of the *Campylobacter* contamination level in broiler batches.

The MALDI method was proved to be quicker and more reliable than the traditional biochemical methods for the identification of *Campylobacter* spp.

The reviewing panel evaluation

A number of interim reports were available to the reviewing panel, which helped them to establish that the majority of objectives to date had been met. This included the monitoring of *Campylobacter* in broiler batches and carcases at national level over time and an evaluation of and validation of laboratory techniques for speciation. There did appear to be some difficulties with the sampling procedures. It was also noted that some data were missing and that there had been some issues with molecular typing. Overall, there had been difficulties in implementing the sampling plan. The panel also questioned whether there had been a 'duplication of effort' between the two parts (A and B) and wondered why they hadn't been asked to work together from the outset (i.e. as one project). From the available documentation and lack of a final report it was not possible to assess if all of the outcomes were successful.

Science: 3 (out of 5)
Value for money: 2 (out of 5)

FS241063

Project title: Investigation into changes of *Campylobacter* numbers on broiler carcasses during and following processing.

Authors: M. L. Hutchison[1,2], S. Gonzalez-Bodi[2], D. Harrison[2], V. K. Morris[2], M. A. Tchórzewska[2], D. Burfoot[3] and V. M. Allen[2]

Institutions: [1]Hutchison Scientific Ltd, Broughty Ferry, Dundee DD5 3EZ
[2]School of Veterinary Sciences, University of Bristol, Bristol BS40 5DU
[3]Campden BRI, Chipping Campden, Gloucestershire, GL55 6LD

Project number: FS241063 (M01055)

Start date: 1st April 2011 **End date:** 31st March 2014

Aims of project:

- Agree a set of standardised sampling and testing protocols with the UK poultry processing industry
- Identify and characterise on a stage-by-stage basis, the processes of slaughterhouses with higher and lower numbers of *campylobacters* on carcasses after chilling
- Identify slaughterhouse with process stages that reduced or increased *Campylobacter* contamination of carcasses
- Survey processing plants to suggest equipment modifications or other interventions to reduce contamination at different processing stages
- Quantify the degree of cross-contamination to an un-colonised flock processed immediately after a *Campylobacter*-colonised flock.

Abstract

A key finding of the EU campylobacters on chicken carcasses baseline survey was that different slaughterhouses have different abilities for the control of contamination when *Campylobacter*-colonised birds are processed. To investigate the basis for the finding, sample collections were undertaken in 18 UK processing plants to identify those with post-chill carcasses contaminated with low or high numbers of *Campylobacter*. The investigations included sample collections in plants and the scrutiny of existing survey and industry data together along with information gathered from a monitoring study (FSA project FS241051).

Overall, 22 processing lines (some plants have multiple lines) were characterised by sampling carcasses after the bleeding, scalding, plucking, evisceration, cropping, inside-outside washing and chilling stages of the slaughter process. Profiles of each process were generated with particular focus on the stages that reduced or increased carcass contamination. While carrying out these measurements, processing operations were observed to identify possible factors that could influence changes in microbial load. Most poultry processors have similar equipment, although during the project duration there was a change from electrical to gas stunning. Gas stunning caused slower exsanguination and, unless lines were extended, caused blood and foam to accumulate in scald tanks. The effect of scalding varied, but tended to reduce the numbers of campylobacters on birds. However, the subsequent plucking and evisceration stages seemed to negate any initial benefit.

The optimisation of water washes was beneficial in one plant where a great deal of effort had been expended on setting up the washers.

Carcasses stained with bile (as an indication of gut rupture during evisceration) usually had higher numbers of campylobacters. The incidence of stained carcasses was low, but higher for larger carcasses.

Chilling consistently reduced the numbers of campylobacters recovered from carcasses. However, most chillers also dry the carcasses to some degree and it was not clear if the reduction was a consequence of bacteria becoming irreversibly bound to drier carcass surfaces or true bacterial death.

As an outcome of these trials, modifications or interventions were suggested to minimise the increase or enhance the decrease in *Campylobacter* numbers on post-chilled carcasses. These included extending the portion of the line used for exsanguination, which reduced post-scald carcass numbers. Optimisation of the inside-outside washer also reduced the numbers of campylobacters on carcasses, but reduced the effectiveness of chilling. A reduction could be achieved either by effective washing or chilling, but not both.

Limited molecular studies were undertaken on isolates collected from the sampling trials to investigate if different strains were influencing the shift in *Campylobacter* numbers through processing. A selection of strains were characterised with no predominant genotypes.

Cross-contamination onto negative flocks appeared a less important factor for controlling campylobacters on carcasses. After 500 un-colonised birds had been processed, there was little indication of cross-contamination from the previously-processed colonised flock.

A conflict of interest was raised by one of the panel because of a previous scientific relationship with the project lead, who was a former undergraduate and post doctorate student. This member did not participate in the review of this project.

The reviewing panel evaluation

Overall this was considered a good piece of work despite the reviewing panel's concerns that it appeared to be repeating what had already been carried out before, especially in other countries. It prompted questions around the politics of using other countries' research. It appeared that the project team completed what they had set to carry out and the quality of the work was scientifically strong. The investigators claim to have identified points in processing of carcasses where interventions could be applied; it would have been helpful if the contractors had hypothesised what these interventions might be. There were some successful outcomes and findings, in particular the surface versus internal *Campylobacter* data collection was considered to be highly useful. The liver surface sterilisation protocols were successful but it would have been useful if the reasons for this had been discussed in more detail. The reviewing panel agreed that the airborne issues in flocks were significant but it would have been useful to have this confirmed by the project team themselves. A criticism was that the project team should have looked at the cumulative effect of interventions in the field. The project would have benefited from mathematical modelling being performed on the data. Based on the findings, it added very little to our current understanding of changes in *Campylobacter* populations on poultry carcasses during processing. This project was potentially held back by using some outdated techniques.

Science: 3 (out of 5)
Value for money: 3 (out of 5)

FS990010

Project Title: Reducing *Campylobacter* Cross-Contamination During Poultry Processing

Authors: Janet Corry [1], Frieda Jørgensen [2], Graham Purnell [3], Christian James [3], Raquel Pinho [1] & Steve J. James [3]

Institutions: [1] Division of Farm Animal Science (DFAS), Department of Clinical Veterinary Science (DCVS), University of Bristol, Churchill Building, Langford, North Somerset, BS40 5DU.

[2] Health Protection Agency, Foodborne Zoonoses Unit (FZU), University of Bristol, Churchill Building, Langford, North Somerset, BS40 5DU

[3] Food Refrigeration & Process Engineering Research Centre (FRPERC)

Grimsby Institute, HSI Building, Europarc, Grimsby, North East Lincolnshire, DN37 9TZ

Project number: FS990010 (M01039)

Start date: July 2005 **End date:** March 2009

Aims of project:

- Identify a 'typical' poultry processing system and any features present in current lines that are not typical but are likely to influence contamination.
- Quantify and identify the main contamination paths in current processing.
- Develop methods of reducing contamination and cross-contamination.
- Evaluate various intervention steps for reducing contamination and cross-contamination.
- Identify the key scientific data that could be used to develop a best practice guide.

Abstract

1. **Six chicken, two turkey and one duck processing line were surveyed** from lairage to portion cutting and staff interviewed. The information gained was used to inform continuing studies of current industrial practice and allow targeting of more detailed experimental evaluative measurements. The results from all chicken plants were further combined into an anonymous description of a 'typical' UK chicken processing plant. There were too few turkey and duck plants to form a representative sample. Where applicable the 'typical' chicken plant was contrasted to the turkey and duck lines. The survey also included an assessment of hygiene, disinfection and cleaning regimes, on which a separate report was written. The effectiveness of the most commonly-used commercial disinfectants was tested against a panel of *Campylobacter* isolates and other bacteria.

The abattoir survey revealed that the techniques used in all chicken processing plants were similar, and that the cleaning the disinfection methods were effective against campylobacters. However, because poultry processing is highly mechanised and is conducted at speeds up to 12,000 carcasses per hour, effective cleaning and disinfection cannot be carried out between flocks, only between shifts – overnight or over the weekend. Also, cross-contamination between adjacent carcasses on the line is unavoidable, and occurs via the machinery and also by direct contact.

2. **Numbers of campylobacters on neck-flaps from *Campylobacter* negative (C–) flocks processed after C+ flocks** were almost always <25 cfu g^{-1}, (160/170 were <25 cfu g^{-1}, seven

between 25 and 99 cfu g^{-1} and three between 100 and 999 cfu g^{-1}) whereas those from neck-flaps from C+ flocks were significantly higher (of 105 examined, two (2%) contained <25, ten (9.5%) between 25 and 99, 49 (46.5%) between 100 and 999 and 43 (42%) 1000 or more).

A further investigation was then carried out to determine whether the first few carcasses from the C- flocks carried higher numbers of campylobacters than those observed in the previous survey. Five neck-skins were examined from the first ~100 carcasses processed, five from carcasses ~500-600 and five from carcasses ~5000-5100 of all flocks processed over several days and from two different poultry plants. Four C- flocks processed after C+ flocks were identified and the numbers of campylobacters per g neck-skin compared with those obtained from carcasses at the same points during processing of C+ flocks. After the first ~100 carcasses, almost all the carcasses from C- flocks had <25 cfu campylobacters g^{-1} neck-skin, while numbers on neck-flaps of carcasses from C+ flocks remained high throughout 28/56 (50%) exceeding 100 cfu g^{-1}, and 10/56 (18%) exceeding 1000 cfu g^{-1}.

Numbers of *Campylobacter* spp. transferred from *Campylobacter* positive chickens to their carcasses during processing. Visits were made by a team of eight to chicken processing plants on five occasions. 10 samples of necks or neck-skins were taken at each of six points on the line during the processing of four flocks at each visit, and numbers of campylobacters, Enterobacteriaceae capable of multiplying at 41.5°C, and pseudomonads were enumerated. Enterobacteriaceae were included as indicators of *Campylobacter* contamination, as they were found in similar numbers in the intestine, and not all flocks were colonised with campylobacters. Pseudomonads were an index of contamination that occurred from the processing environment. Most carcass contamination with *Campylobacter* spp. and Enterobacteriaceae was detected after scalding with little obvious increase after plucking, or after evisceration. Contamination with pseudomonads increased steadily all down the line after scalding.

In order to clarify which process was the most important source of carcass contamination with campylobacters, batches of chicken carcasses were removed from the line immediately after plucking and dipped in water at 80°C for 20 s before replacement on the evisceration line. Control carcasses (processed normally) were taken after evisceration, as well as carcasses that had been decontaminated with hot water after plucking. All were sampled by examination of neck flaps or necks and the carcass rinse method. Results showed that plucking and evisceration contributed to a similar degree to numbers of *Campylobacter* spp. and Enterobacteriaceae on the fully processed carcasses.

3. **An extensive literature review, brain-storming sessions and discussions were held in order to identify the methods most likely to be successful in reducing numbers of campylobacters on carcasses from *Campylobacter*-positive (C+) and *Campylobacter*-negative (C-) flocks.**

With C+ flocks the problem is to try to minimise transfer to the finished carcass, of campylobacters present in high numbers in the intestinal contents and on the feathers of the birds. Practical investigations in processing plants showed that similar proportions of contamination was occurring at two main points - during the scald and pluck stage, and subsequently during the evisceration steps. It was therefore concluded that reducing significantly the numbers of campylobacters reaching the carcasses during scalding and plucking would be of little benefit if subsequent processing steps during evisceration contributed a similar number. However, a better system for scalding and plucking and/or cleaning and disinfection all along the line between flocks might be effective in reducing cross-contamination from C+ to C- flocks. However, the studies conducted under Objective 2 showed that there was little cross-contamination from C+ to C- flocks beyond the first few hundred carcasses.

The other clear possibility was to investigate the effect of end-product treatment of the fully-processed carcasses, either immediately before, during or after chilling. The possibilities were to use physical (e.g. steam at atmospheric pressure or dipping in hot water) or chemical (e.g. chlorine, chlorine dioxide (CD), acidified sodium chlorite (ASC), ozone, trisodium phosphate (TSP), mixtures of peroxy acids (PAA)).

4. **Naturally contaminated *Campylobacter*-positive chicken carcasses, obtained from a commercial processing line immediately post-evisceration but prior to the inside-outside wash, were treated in a purpose-built automated spray rig**.

Replicated batch treatments for 15 s and 30 s of chemical and water only spray wash were performed. Untreated control carcasses were examined to provide baseline data for the initial numbers. Numbers of colony-forming units (CFU) per g of skin excised from neck and breast locations were determined using selective agar media.

For analysis, the results were subdivided into six microbe-type/skin-location combinations with each subdivision ranked by: a) CFU remaining after treatment, b) mean reductions, and c) the proportional change in numbers of samples below the limit of detection (LoD).

The three groups of bacteria responded similarly to the chemicals applied, with maximum reductions of 1 – 2 log cycles. *Campylobacter* spp. were no more susceptible than the other two groups. Generally ASC and TSP were more effective in reducing microbial counts than PAA, with CD and water having the least effect. A 30 s chemical treatment was usually more effective than a 15 s treatment. Where only a short (15 s) spray time is possible, ASC appears the most effective. Where longer treatments are possible, TSP becomes the most effective, but has environmental drawbacks. Similar reductions had been obtained in a previous FSA project, using steam at atmospheric pressure or dipping in hot water.

The reviewing panel evaluation

This project involved a large literature review of a number of different stages along the processing line. The particular focus was on interventions in reducing *Campylobacter* contamination on the carcass. The panel agreed that lots of useful information had been identified but a drawback was that some of it was sourced from out of date literature. The work on decontamination techniques was particularly insightful although the mechanism for rating the interventions was somewhat subjective. The costs were considered reasonable given the task required. Subsequent publication of the already delayed final report is unlikely to have the desired impact given the older literature sources and the rapid advancement in technology. Value diminishes if the findings from the report are not used therefore it is difficult to be absolutely accurate with the value for money score at this stage. It was also noted that the principal investigator left the university part way through the project. Reviewers questioned the governance of this work, stating that the FSA should make reasonable attempts to ensure that key members of the project team remain in post for the duration of the project where possible.

Science: 3 (out of 5)
Value for money: 3 (out of 5)

FS121014 E

Project Title: CampyTools project 'A comparison of the UK slaughterhouse Hygiene Tool & DI Tool developed in Belgium in terms of impacts on *Campylobacter* levels' & 'Comparison of FSA and DI Tool to measure performance of current food safety management systems in poultry slaughterhouses with special reference to *Campylobacter*.'

Authors: Arie Havelaar[1], Ewa Pacholewicz [1], Lieven De Zutter[2], Mieke Uyttendaele[2], Liesbeth Jacxsens[2], Julie Baré[2], Tomasz Seliwiorstow[2], Toby Knowles[3], Mike Hutchison[3], Monica Tchorzewska[3], Viv Allen[3]

Institutions: [1]University of Utrecht
[2]University of Ghent
[3]University of Bristol

Project number: FS121014 E

Start date: February 2013 **End date:** July 2014

Aims of project:
This was a comparison of two tools: 'FSA tool' (detailed technical tool) and FSMS-DI' (generic indicator tool) to measure practices in poultry slaughterhouses and performance of Food Safety Management Systems.

The reviewing panel evaluation

The FSA tool was a scoring system for poultry-plants, based on current best scientific evidence, of the hygiene practices likely to impact on *Campylobacter* load on carcasses. The FSMS-DI tool was more context-based that included some hygiene procedures but also broader detail including supply chain, integration and QA processes. It was concluded that the FSMS-DI tool was superior given that it took into account the integrated safety mechanisms. It also demonstrated value and good technical quality. The reviewing panel did question their rate of implementation and uptake. Overall, it was concluded that neither model as a standalone was particularly accurate at predicting *Campylobacter* levels post chill, however when used in combination, it did allow industry to make some comparisons, thus instilling a little more confidence in the outcomes if the results were similar in both models.

Science: 4 (out of 5)
Value for money: 4 (out of 5)

FS101038

Project Title: Investigation of the efficacy, practicality and cost effectiveness of modified atmosphere packaging on *Campylobacter* numbers on raw chicken intended for retail

Authors: Dean Burfoot[1], Lynneric Potter[1], Craig Ballard[1], Michael Bonin[1], Keith Jewell[1], Alan Campbell[1], Victoria Morris[2], Mike Hutchison[2], Dawn Harrison[2], Monika Tchórzewska[2]

Institutions: [1]Campden BRI
[2]University of Bristol

Project number: FSA Project FS101038

Start date: 1st February 2013 **End date:** 31st March 2015

Aims of project:

- To collate information from gas suppliers, the poultry industry, and scientific literature on the gas mixes currently in use and their effects on *Campylobacter*
- To define a control for comparison with the effect of MAP (air in high or low permeability film) and incubation temperatures for microbiological testing of aerobic plate counts.
- To measure gas:meat ratios.
- To quantify the effects of microbial counts, drip, colour, rancidity and odour of gases currently used by the poultry industry.
- To assess the variation in *Campylobacter* counts around MAP carcasses.
- To identify the optimum gas mix.
- To carry out temperature abuse testing and full scale trial.

Abstract

- Collation of information from industry showed that whole birds are packed in 70 or 80% oxygen and 30 or 20% carbon dioxide or they are packed in air. Five gas mixes, one being 80% O_2/20% CO_2, are used for packing portions. A review of the scientific literature indicated that the 80% O_2/20% CO_2 mix is at, or close to, the optimum to reduce *Campylobacter*. The studies used inoculated birds.
  After packing birds in air, packs with higher permeability film resulted in lower numbers of *Campylobacter* on the birds. No statistically significant evidence was found of an effect of incubation temperature (20 or 30˚C) on aerobic plate counts.
- Packs of whole birds obtained directly from poultry producers had gas:meat ratios of 0.5; other ratios were used for portions.
- Whilst discussions were underway regarding the protocols for Objective 4, trials under Objective 5 found higher counts on back skin compared to breast skin but no effect of modified atmosphere (80% O_2/20% CO_2 versus air) on numbers of *Campylobacter*.

Objective 4 was revised to replace one trial including measurements of quality with three trials measuring gas mixtures and numbers of *Campylobacter*. Packs starting with 70 or 80% oxygen showed wide variations in oxygen concentration after three days of storage: between about 80% and 20% oxygen in packs starting with the same gas mix. This variation might result from many factors including microflora, pack leakage, variations in flushing, and bird conformation and trim. *Campylobacter* numbers on the birds were low in all trials, being generally below 1.5 log when tested at three days after kill and packing (K+3). There was only weak evidence of an effect of MAP on numbers of *Campylobacter* at Day K+3 ($p=0.04$). Differences between these trials and those reported in the literature include using (a) naturally contaminated birds, (b) different films for packing birds in air or MAP, (c) low gas:meat ratio, (d) large variations in gas mix during storage, (e) testing at Day K+3, (f) low numbers of *Campylobacter*.

The FSA put the project on hold in July 2014 and stopped it in early 2015.

The reviewing panel evaluation

This project was terminated, which was the appropriate action to take according to the reviewing panel. This was partly due to sampling giving rise to low levels of campylobacters to test, and also that early findings concluded that MAP was of only minor, if any value, in *Campylobacter* control. It was noted that the contractors did carry out a large number of experiments and met the first five objectives before the project was terminated. This project was also praised for trying to carry out investigations within a real life setting. It was noted that previous work had not been conducted within a similar setting (the spiking work). The study was undermined by a small number of samples. It had unfortunately encountered difficulties with natural *Campylobacter* on chickens and the bleeding of gas mixtures from the MAP packs. It was concluded that this project was of moderate value and was perhaps something for the Agency to look into again. The project outcomes gave a clear message that MAP is of only minor, if any, value in *Campylobacter* control.

The review board questioned the governance of this project, and felt that perhaps the wrong question was cited in the original specification of this work. Rather than focus solely on MAP, the reviewing panel agreed that the FSA should have asked ‘What is it that is making *Campylobacter* levels decrease post packaging?’ This would have increased the scope of the project. Under the current scope, the contractor had done all that they could do in the circumstances. If the report is to be published, the reviewers felt that some care needed to be taken in the drafting of this to ensure that the outcomes do not necessarily assume MAP is not effective, it could be that MAP would reduce levels if packaging materials were less permeable.

Science: 3 (out of 5)
Value for money: 3 (out of 5)

FS241044

Project Title: A UK wide microbiological survey of *Campylobacter* contamination in fresh whole chilled chickens at retail sale.

Authors: Frieda Jorgensen[1], Robert H Madden[2], Eve Arnold[1], Andre Charlett[1] and Nicola C Elviss[1]

Institutions: [1]Public Health England
[2]AFBI

Project number: FS241044 (includes brief update on FS102121 which is the follow-on study)

Start: February 2014 and July 2015 **End:** July 2015 and to June 2018

Abstract
In order to ascertain the number of campylobacters on retail chickens this survey tested 4,011 whole, UK-produced, fresh chickens from February 2014 to March 2015. Sample purchases were evenly distributed throughout the year and the UK (in proportion to the population). Retailers were sampled in proportion to their market share, according to available data, with the share of free-range and organic chickens taken into account. The chickens were examined using the EN/TS/ISO 10272-2 standard enumeration method (applied with a detection limit of 10 cfu per g of skin or per outer packaging swab sample tested).

Across the 12-month of the survey the prevalence of *Campylobacter* spp. in fresh chickens at retail in the UK was 73.3 %. A significant proportion (19.4 %) of samples had >1000 cfu/g of chicken skin and in 6.8 % of samples campylobacters were detected on the outer packaging. The *Campylobacter* spp. contamination found on the outer packaging was mostly at low levels, but levels of between 100 and 4,500 cfu of campylobacters per pack were detected in 1.6 % of samples. There was a significant difference in the proportion of chickens with >1000 cfu/g, based on the approval code/slaughter house premises mark. A larger proportion of chickens had a high level of *Campylobacter* spp. during the summer (compared to winter months). Larger chickens (i.e. those > 1400 g) were more likely to be contaminated with > 1000 cfu/g. There was no evidence that birds with access to range (e.g. free-range and organic birds) were less contaminated than birds reared under standard conditions but no precise comparison could be made. For the campylobacters from chicken skin which were speciated, most (76.6 %) were *C. jejuni*. *C. coli* comprised 13.9 % and both species were found in 4.2 % of samples. *C. coli* was more frequently isolated in the summer compared to winter and spring months and was more frequently isolated from birds with access to range. From the isolates obtained 230 *C. jejuni* and 53 *C. coli* had their antimicrobial susceptibility determined and these will be published as a separate report after peer-review.

Results from the 1,998 chickens sampled in the first six months of a second survey year (July to December 2015) showed that 67.1 % of skin samples were positive for *Campylobacter* spp. and 12.6 % showed levels of > 1000 cfu/g (without data weighting). Comparison of data from the first and second survey year has demonstrated that the prevalence of highly contaminated chickens was significantly lower in the second survey year compared to the same sampling months in the previous survey year.

One of the panel had a conflict of interest as their company was subject to sampling of fresh whole chickens in this research. This member did not participate in the review of this project.

The reviewing panel evaluation

Overall it was felt that an excellent report was produced, which fully captured the science around the testing procedures and was well written. Despite this, there did appear to be some issues with its validity. It was felt that this project had been steered from a political view point to generate information on retailer prevalence rather than overall prevalence of *Campylobacter* on chickens. This could unwittingly give consumers a false sense of security over certain retailers' chickens. This 'political' driver had come from the FSA specification rather than the contractor, however, it was noted that the key findings (of prevalence) were similar to previous 'non-political' *Campylobacter* surveys.

Of the findings, the packaging contamination data were particularly informative given that not much work had been carried out previously in this area. There were a number of concerns raised around the sampling protocol. Although the project incorporated the latest market share data from Kantar at the time (from 2010), it was felt that it didn't reflect accurately the current market share at the time the survey was carried out. A reference was made to the rapid rise in the popularity of discounters. Reviewers commented that this work was surveillance rather than a research project per se, and as such, needs to be carried out continually with the same methodology to get robust data that can be compared over time.

A new survey is currently underway (FS102121), which has incorporated updated market share data. Another concern raised was that there weren't any conclusions drawn from the prevalence of *Campylobacter* in organic chicken. This was due to a much smaller sampling number than housed broilers. There were also some concerns raised about why the project was commissioned and its general purpose.

Science: 4 (out of 5)
Value for money: 4 (out of 5)

FS121014 C

Project Title: Maintaining sentinel surveillance for human Campylobacteriosis in Oxfordshire: monitoring the impact of poultry industry interventions on the burden of human disease.

Authors: Martin Maiden[1]

Institutions: [1]University of Oxford

Project number: FS121014 C (MOU with Defra for 50% funding, Defra-led)

Start date: August 2011 **End date:** August 2014

(Jul 2011 to 30/06/14) FSA-Defra joint MoU. Cont'd as **FS101119** FSA sole funded (1/7/14 to 31/12/14).

Aims of project:
This project will continue the epidemiological surveillance of Campylobacteriosis in Oxfordshire, which has been ongoing since 2003. The 2003 to 2010 data will be used as a baseline, and compared with the data gathered in this project to see if an effect of interventions designed to control *Campylobacter* in the poultry industry can be observed.

The reviewing panel evaluation

This was a Defra-led project, whereby FSA allocated 50% of the funding. It was known that this work had been carried out and completed. However, due to insufficient and incomplete documentation available to the reviewing panel, it was not possible for an evaluation to be made.

Science: Evaluation not carried out; no score assigned
Value for money: Evaluation not carried out; no score assigned

FS101025

Project title: Freezing as an intervention to reduce the numbers of campylobacters isolated from chicken livers

Authors: D. Harrison, J. E. Corry, M. A. Tchórzewska, V. K. Morris, M. L. Hutchison

Institutions: School of Veterinary Sciences, University of Bristol, Langford, Bristol BS40 5DU

Project number: FS101025

Start date: 1st October 2012 **End date:** 31st March 2013

Aims of project:
The aims of this study were to determine the prevalence and numbers of campylobacters in 63 samples of raw livers purchased at retail across the UK. In addition, whether the freezing of final clearance chicken livers contaminated with *Campylobacter* was investigated to determine if the process was a reliable method for the decontamination of livers.

Abstract
Chicken livers were sourced from final clearance flocks to maximise the chances of collecting livers from birds that were naturally colonised with campylobacters. Contaminated livers were subjected to freezing treatments of -15°C and -25°C for one day and seven days. The numbers of campylobacters on the livers were determined immediately before and after a 24 h or 7 d freeze treatment and daily during a three day, post-thaw refrigerated storage. Freezing for 24 h at -25°C caused reductions to campylobacters of up to two logs. Freezing the livers for 24 h duration at -25°C, thawing overnight in a fridge set to 4°C and refreezing for another 24 h to -25°C caused reductions to the numbers of campylobacters of up to three logs. There were significant reductions in the numbers of campylobacters when the single and two stage freeze treatments were compared. Freezing chicken livers can reduce, but not eliminate, campylobacters. If poultry processors were to freeze livers destined for human consumption as part of routine processing, there is a potential for a reduction in the foodborne illness associated with the consumption of imperfectly cooked chicken livers and processed derivatives, such as pâté.

The reviewing panel evaluation

The reviewing panel felt that this was a good, small project, whereby the work produced was very compelling and at a low cost. Important data were collected and a substantial amount of microbiological work was carried out, which has helped this project to be great value for money. It successfully demonstrated the effect (log reduction) that freezing had on *Campylobacter* populations in raw livers. This helped to support the practical benefits of freezing chicken livers to reduce *Campylobacter* levels. Overall, the project delivered well and it was judged to have fully met its objectives. More clarification was needed on where the highest levels of Campylobacter were in the livers sampled. It was concluded that the outcomes could be used further i.e. it could point to a practical industry intervention that all livers are pre-frozen to significantly minimise the risk.

Science: 5 (out of 5)
Value for money: 5 (out of 5)

FS101062

Project title: Controlling *Campylobacter* during the manufacture of chicken liver pâté

Authors: M. L. Hutchison, D. Harrison, I. Richardson and M. A. Tchórzewska

Institutions: School of Veterinary Sciences, University of Bristol, Langford, Bristol BS40 5DU

Project number: FS101062

Start date: 1st October 2013 **End date:** 31st March 2014

Aims of project:
The aims of this study were to devise a protocol for the preparation of commercial quantities of pâté that reliably destroyed any campylobacters contaminating the chicken liver ingredient. Prior to the commencement of the study, a number of large outbreaks at commercially-catered functions (e.g. weddings) had occurred because caterers had been undercooking livers in an attempt to keep the pâté pink and appealing to consumers.

Abstract
A literature search of internet recipe sites and traditional recipe books identified 40 pâté manufacture recipes. Recipes were appraised and stages that were likely to be antimicrobial were assembled to form a new protocol that included washing with non-brewed condiment (5% w/v ethanoic acid) or 5% w/v lactic acid, freeze-thaw and a flambé in alcohol. Contaminated livers from organic clearance flocks were obtained directly from slaughterhouses as high-risk material and the effect of each stage of the protocol to *Campylobacter* populations on naturally-contaminated livers was determined. Typically, washing with either of the organic acids bleached the colour of the liver surfaces. However, there were no significant differences between liver surface colour changes when a range of concentrations of lactic acid and ethanoic acid washes were compared by reflective spectrophotometry. Bleaching was confined to the external liver surfaces. Both of the organic acid washes reduced numbers of indigenous campylobacters by around 1.5 log10 CFU/g. Liver cooking effectiveness was appraised by infra-red thermography and temperature loggers. The use of a Bain Marie was found to more reproducibly apply heat compared with pan-frying. The antimicrobial protocol stages reduced the numbers of campylobacters, but not significantly if thermal processing was ineffective. Cooking to 63°C was confirmed to be a critical control point for campylobacters in pâté. Organoleptic and sensory assessment of pâté manufactured using the protocol from fresh or frozen livers determined both to be palatable, with an overall preference for pâté made from frozen livers.

The reviewing panel evaluation

This was a short and inexpensive project which had successfully executed what it had set out to achieve. It was an excellent piece of applied research, with credit given that a scientific publication in the International Journal of Environmental Research and Public Health was released as part of the work. There was a missed opportunity as it didn't prominently feature on the FSA's website. This project was referred to as an excellent example of FSA funding which has led to the production of a 'safe' method for preparing a high risk food. It was felt the FSA could do more to publicise this recipe, especially to industry, given the popularity of chicken liver pâté.

Science: 5 (out of 5)
Value for money: 5 (out of 5)

FS241040

Project Title: Development of accurate predictive models for the assessment of the survival of *Campylobacter jejuni* and *C. coli* under food-relevant conditions.

Authors: Andrew Close[1], Trevor Jones[2], Tom Humphrey[3], Steven Rushton[1], Nicola J Williams[2]

Institutions: [1]School of Biology, Ridley Building, Newcastle University, Claremont Road. Newcastle Upon Tyne, NE1 7RU.
[2]Liverpool University
[3]Swansea University

Project number: FS241040

Start date: 1st April 2011 **End date:** 31st January 2015

Aims of project:
The aims of this project were to assess *Campylobacter* survival in foods and food related environments in the literature, to determine the behaviour of *C. jejuni* and *C. coli* in laboratory media, when associated with, or inside poultry muscle meat at sub-lethal and high temperatures and during sous vide cooking of whole breast meat and to develop predictive models to predict such survival.

Abstract
Campylobacter are major zoonotic pathogens and control in the food chain is a public health necessity. One approach is to use potentially lethal processes such at heating and cooking to reduce the threat. *Campylobacter* is said to be sensitive to hostile environments, but it survives in food products that have been refrigerated and/or heated prior to consumption. Predictive models may help food processors identify appropriate treatments to eliminate pathogenic bacteria like *Campylobacter*. However, data currently available are inappropriate as experimental techniques do not take into account interactions between *Campylobacter* and foods, or recognise the population *biology* of *Campylobacter* and variation in heat resistance between strains.

A review of the literature found little in the way of conformity with regard to the species and strains used, or methodological approach used during experimental studies. Furthermore, not all studies have explored the effects of selective and non-selective media on the recovery of sub-lethally damaged *Campylobacter* cells. Heterogeneity in experimental design with regard to combinations of enumeration media used and the variability in temperature and temporal profiles are such that a synthesis of methods and findings for the purposes of formal meta-analyses was not possible. Standard operating procedures were developed for all experiments, with a comparison of both selective media, namely modified charcoal cefoperazone deoxycholate agar (mCCDA) containing the selective supplements and the non-selective Columbia blood agar containing 5 % defibrinated horse blood (CAB) with *Campylobacter* growth supplement (FBP), which contains oxygen quenching agents, to allow a comparison between the recovery capabilities of the two media, and to assess the recovery of sub-lethally damaged cells on the latter media at 56°C. Significant differences in the numbers of *Campylobacter* cells recovered were found between the two types of media, although differences were strain specific with increased numbers of sub-lethally damaged cells recovered from non-selective media for strain 11168 only during the midpoint of the simulation. This indicates that extended exposure to heating at 56°C is likely to have resulted in the removal of sub-lethally injured cells, leaving a final sub-population of viable cells, whose growth is not enhanced by the use of non-selective CAB/FPB media. These findings indicate that the use of selective media for such experiments may negatively affect the recovery of sub-

lethally damaged cells and underestimate the survival of *Campylobacter* undergoing heat treatment. Experiments were undertaken to examine differences in the underlying survival rates of *Campylobacter* strains in pre-heated laboratory media at different temperatures (56°C, 60°C and 64°C) and pH values (4.5, 5.5, 6.5, 7.0 and 8.5). Differences between strains of *Campylobacter* were observed with respect to the survival of each strain and the degree of sub-lethal injury to cells. Variation was noted in underlying survival curves between isolates of the same clonal complex as well as between clonal complexes. Examining the impact of both pH and temperature demonstrated that strains are able to survive well at lower pH ranges, with optimal survival for one strain (12662, CC-257) at pH 5.5 even when challenged at 64°C. Thus, *Campylobacter* may not be as sensitive to acid pH as previously thought.

Campylobacter survival was enhanced at sub-lethal temperatures (56°C) when in association with poultry meat. However, at higher temperatures (60, 64, 68 and 70°C) attachment had no impact on *Campylobacter* survival. Given that meat will be pre-chilled before cooking, chicken inoculated with *Campylobacter* was subject to overnight chilling in a fridge (4°C) and then subjected to direct heating at 60, 64, 68 and 70°C. The impact of pre-chilling varied between strains but, due to insufficient reproducibility of experimental replicates at higher temperatures, it could not be determined whether such differences were truly significant.

As whole portions of meat are often subject to gradual heating, small pieces of chicken meat were inoculated on their surface with *Campylobacter* and subjected to gradual heating in a water-bath. After reaching the target temperature of 70°C (after 16 minutes), the temperature was then held for six minutes resulting in a large reduction in counts (Log_{10} 2.7-4.2) for all tested strains, however *Campylobacter* could still be detected intermittently during this time, suggesting very low levels at the limits of microbiological detection/recovery. In addition, direct heating of chicken meat internally contaminated with high levels of *Campylobacter* (~10^6 CFU) at high temperature (68°C internally) also demonstrated that a residual population was present up to 14 minutes after being kept at this temperature. Whilst high numbers were inoculated into meat, these results have implications in terms of the FSA recommendations concerning 70^{oC} at two minutes being adequate for removing all viable *Campylobacter*.

Sous vide cooking of meat has become very popular in recent years and using this method to cook whole artificially inoculated chicken fillets in a commercially available cooker at low temperature (50-52°C) demonstrated *Campylobacter* survival even after three hours of cooking at 50^{oC}. However, chicken meat appeared under-cooked (raw) after such cooking times. At 56°C the meat did not appear under-cooked after cooking for one hour and for one replicate of three, a *C. coli* strain could still be detected at 1 log CFU, indicating for some strains that this temperature for one hour was inadequate to kill all *Campylobacter* present in the fillet.

Selecting an appropriate non-linear function to generate predicted response curves that best describes the response of *Campylobacter* is a complex task. The manner in which *Campylobacter* responds to challenge may be a combination of strain characteristics and also the magnitude and type of challenge used during experimental simulations. Our findings indicated that no single model was adequate under all circumstances and that the selection of an appropriate model should be based on visualising the type and shape of survival curve generated by experimental data. However, a general model prospoed by Coroller *et al*. (Appl.Environ.Microbiol.2006, 72(10) 6493-6502) was identified as a possible solution to this problem. A GlnaFiT (1.6) package was also developed by Geeraerd *et al*. (Int. J. Food. Microbiol.2006.102 (1) 95-105) and this provides a user friendly means of fitting and evaluating ten different models capable of generating predicted response curves, including the general Weibull model proposed by Coroller *et al.* (2006).
This software is freely available and maintained by University of Leuven: http://cit.kuleuven.be/biotec/downloads.php. A user manual has been produced to assist with users in selecting appropriate models as part of this project.

Gradual heating of chicken meat pieces inoculated externally and internally to 70oC has demonstrated survival of low numbers of *Campylobacter*, which is of concern. Given that retail surveys have found levels of up to 105 CFU/g contamination of *Campylobacter* on carcasses, these results may suggest that the current recommendation of 70°C for 2 minutes may be inadequate in inactivating all *Campylobacter* present in meat when contaminated at such levels. However, more work is required to investigate if such sub-populations of viable cells are still able to cause infection and therefore represent a significant public health threat. Further work should also be undertaken to elucidate the mechanisms involved in the survival of these residual populations and their association with meat surfaces.

Finally, our findings indicate that no one single model describes the survival of *Campylobacter* under all circumstances, and that the selection of an appropriate model should be based on individual strain and response to type and magnitude of challenge used during experimental simulation.

A conflict of interest was declared by one of the panel members. The panel member was the project investigator from the outset but had no involvement with the delivery of the project.

The reviewing panel evaluation

The panel's evaluation was that this project had achieved the majority of a long list of objectives. A significant amount of work had been carried out, with a large proportion of this being experimental work. For this to have worked, the project team needed a robust data set, which was lacking.

A huge amount of data was generated over the course of this project. Individual models were produced for the various analyses but no attempt was made to analyse across the various experiments and test the extent to which a robust model could analyse larger amounts of data generated across experiments, nor test discrepancies in the data across different experiments. There were concerns with the methodology used as there appeared to be a number of interferences with heat. The work should have been presented in the context of comparisons with other studies rather than a list of conclusions, some of which have huge implications.

In relation to this, the panel strongly encouraged the sharing of data, which would have been highly advantageous for this piece of work. Validation on the work carried out, in particular on surviving cells, was another suggestion.

Science: 2 (out of 5)
Value for money: 2 (out of 5)

FS101042

Project Title: ENIGMA: Sources, seasonality, transmission and control: *Campylobacter* and human behaviour in a changing environment

Authors: Sarah O'Brien[1], Peter Diggle[2], Nicola Williams[1], Paul Hunter[3], Dan Rigby[4], Steve Rushton[5] on behalf of the ENIGMA Consortium

Institutions: [1]University of Liverpool,
[2]Lancaster University,
[3]University of East Anglia,
[4]University of Manchester,
[5]Newcastle University

Project number: Grant Reference: G1100799/1 (FS101042)

Start date: 1st January 2012 **End date:** 31st December 2016

Aims of project:

- Identify the key reservoirs, environmental and social drivers of *Campylobacter* that affect human disease;
- Analyse seasonal variations in pathogen load and their impacts on exposure and disease;
- Understand the relative roles of the transmission pathways and thus points of control;
- Generate future projections of disease risk and its control.

Objectives

1. Identify how campylobacters persist in the natural environment and how environmental exposure influences their virulence; analyse the interaction between *Campylobacter* populations and their environment; estimate the direct and indirect contribution of environment and human behaviour on the risk of human *Campylobacter* infection (Aim 1).
2. Analyse spatial and temporal variation in *Campylobacter* load in contrasting rural environments to quantify seasonal variation in human exposure to *Campylobacter* (Aim 2).
3. Analyse the relative importance of different transmission pathways (recreation, water, food, etc.) to humans; analyse risk perceptions of rural environment users; analyse effectiveness, acceptability, costs and benefits of interventions to reduce burden by estimating the current costs of *Campylobacter* disease; analyse interventions and assess their acceptability (Aim 3).
4. Predict changes in disease burden due to environmental and/or social change; predict how future changes in climate, land use, countryside visits, food production and consumption will affect disease patterns and costs (Aim 4).

Abstract

Diarrhoeal disease is an important global killer that causes major health and economic problems. Many organisms that cause it are zoonotic. They are widely distributed in the environment and there are several pathways to human disease.

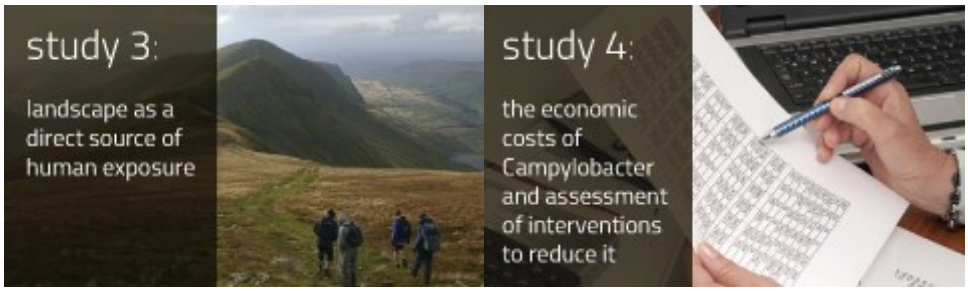
Research into diarrhoeal disease to date has been largely biomedical and focused mainly on transmission through contaminated food or water. Fundamental gaps in our knowledge

remain, namely the contributions of human behaviour and human-environment interactions influencing exposure to organisms and risk of disease. We have brought together scientists from a wide variety of disciplines in a “one health” approach to tackle these fundamental knowledge gaps using *Campylobacter spp.* as an example. Important in its own right, *Campylobacter* is the most common bacterial cause of diarrhoeal disease in the developed world. It caused an estimated 700,000 cases in the UK in 2010 with ~200 deaths. Extreme outcomes include irritable bowel syndrome, arthritis and paralysis.

The current, underestimated, annual UK cost of *Campylobacter* infection alone is £600m, exceeding that from Salmonella, Listeria and *E. coli* O157 combined. The transmission pathways for ~50% of human cases are unknown. Seasonal dynamics are central to the disease burden because ~40% of cases occur during the ‘spring peak’, yet the relative roles of environmental and food pathways, and their interaction, in this seasonal emergence are poorly understood.

To make major progress we face two important challenges. The first is to develop new methods that incorporate environmental and social systems to understand how they interact with *Campylobacter*. Secondly, since the behaviours of both humans and *Campylobacter* involve processes that play out over different temporal and spatial scales, the new methods we develop need to capture this. The challenge of analysing systems and data at different scales, whilst minimising loss of information in so doing, will be generally applicable to research on the interaction of social and ecological systems and zoonoses.

The work is organised into five interlinked, interdisciplinary work packages:



Dissemination to date

CHRO 2015

The 18th International Conference on *Campylobacter*, Helicobacter and Related Organisms (CHRO) was held from 1st–5th November 2015 in Rotorua, New Zealand. Members of the Enigma study attended the conference and shared research findings with scientists from around the globe. The conference featured:

Plenary Lecture:

- *Campylobacter*: epidemiology of an enigmatic organism (Prof Sarah O'Brien)

Oral presentations:

- Survival of *Campylobacter jejuni* in soil from the dairy farm environment is dependent upon strain, temperature and presence of other micro-organisms.
- *C. jejuni* M1 survival in nutrient poor water: gene expression profiles during the viable but non-culturable (VBNC) state
- A longitudinal study of *Campylobacter* in a dairy farm environment.

Poster presentations:

- Population SNP profiling (PSP) of *Campylobacter jejuni* from the poultry and dairy farm environment
- Survival of *Campylobacter* in the Poultry Farm Environment
- Molecular typing of *Campylobacter* isolated from the Poultry Farm Environment
- *Campylobacter* in children under the age of five years old
- Exploring porA allele diversity among *Campylobacter jejuni* isolated from the environment on a dairy farm.

A conflict of interest was declared by four panel members. These members did not participate in the review of this project.

The reviewing panel evaluation

This project is still ongoing therefore the panel felt it was too soon to look at any outcomes. However, it was commented that the work carried out to date included high quality science and the project outcomes would produce some very useful information and data. Overall, the scope of the project was highly praised and what was particularly impressive was the social science input. The panel were also complimentary about the multidisciplinary nature of the work, which will help to encourage the transferring of relevant science. Yearly updates are provided via Research Fish, which the FSA does not currently have access to. The FSA confirmed that they would consider if this would be a useful feature to have access to going forward.

Science: 5 (based on early reports) **Value for money:** A score could not be awarded at this stage.

FS421003

Project title: Employing source attribution and molecular epidemiology to measure the impact of interventions on human Campylobacteriosis in Scotland.

Authors: Ken Forbes[1], Norval Strachan[1]

Institution: [1]University of Aberdeen

Project number: FS421003

Start date: Nov 2010 **End date:** Jan 2016 (subsequently FSS from 1st April 2015)

Aims of project:

- Collect and archive clinical, cattle, sheep, pig and retail poultry (chicken, turkey) *Campylobacter* isolates in Grampian.
- Undertake MLST.
- Undertake the molecular characterisation of source and clinical *Campylobacter* isolates and the attribution of human cases to the sources.
- Compare results with previous studies to determine whether/how the attribution to different sources has changed over time.

Abstract

This study identifies continuing extensive population diversity of *Campylobacter* strains in farm animals, in wild birds, in retail poultry and in human isolates. The relative abundance of the strain types found in these reservoirs continues to be dynamic with even the relative abundance of the commoner strains changing between the 2005-07 study and this 2012-15 study, indeed even over periods as short as one year. Notwithstanding this, the strain profiles in each reservoir species are characteristic of that particular host, and thus the basis of molecular attribution modelling continues to hold. The attribution models and associated datasets were validated by self-attribution testing.

Host attribution modelling of putative sources of human infection suggests that there continues to be broadly the same proportional attribution over all the study periods since 2005 with retail chicken being the largest contributor of *Campylobacter* infections, followed by cattle, sheep, turkey and other less common sources.

From April 2012 to March 2015 the source attribution modelling allocated clinical isolates to the following reservoirs: poultry 52-76%, cattle 9-10%, sheep 9-20%, pigs 0-8% and wild birds 3-5%. The inclusion of strains from turkey in this study has identified another contributor source for human Campylobacteriosis. However, molecular attribution scores are principally a measure of probability of human case strains having come from different proportions of the tested reservoirs with a component of the attribution deriving from the likelihood of exposure (contact) of humans to each source. For most people their contact with farm animals or with wild birds, or of their consumption of contaminated turkey meat will all be rare. We have shown that urban children have a lower incidence of ruminant attributed strains and that there is no spike in turkey attributed cases in December of each year - a popular festive meat at this time. Retail poultry, principally chicken, remains as the largest contributor of *Campylobacter* infections in Grampian, and therefore also in Scotland.

The current study provides insights into the dynamic nature of *Campylobacter* and provides key baseline data on prevalence and strain types in the main food vehicles and animal reservoirs.

A conflict of interest was declared by one of the panel members. This member did not participate in the review of this project.

The reviewing panel evaluation

This was a well written piece of work. It involved the setting up of an archive, and it was felt very important to have this resource. A number of objectives were met including collection and archival of human clinical, cattle, sheet, pig and retail poultry isolates of *Campylobacter* in the Grampian region. This project significantly improved understanding of source attribution in attributing transmission of *Campylobacter* infection in the food chain. Poultry meat was the largest reservoir for human isolates but a smaller, significant proportion was still identified in sheep and cattle and wild birds. The project outcomes were considered highly beneficial. Overall, it has shown that the work was of high quality, yielding some very important data. The panel were confident that either referring to, or using, this data could be relied upon. This project was held up as an excellent example of funding by the FSA.

Science: 5 (out of 5)
Value for money: 5 (out of 5)

BBSRC led grants (FSA part funded)

These projects were commissioned as part of the Joint *Campylobacter* Strategy, working with Defra, and FSA on *Campylobacter*. The research element was funded by BBSRC. The translation research was funded by FSA & Defra. Of the programme only two grants were solely funded by BBSRC. All completed work was also peer reviewed. BBSRC did not insist on industry co-funding, but they did request industry input. Strategic and policy relevance was a key assessment criterion.

FS231081

Project Title: How production systems, bird welfare and endemic disease affect the susceptibility of chickens to *Campylobacter*.

Authors: Dr Paul Wigley[1]

Institutions: [1]University of Liverpool

Project number: FS231081 (BB/I024674/1)

Start date: November 2011 **End date:** April 2014

Aims of project:
This research examined the different systems in which UK chickens are grown to identify cost-effective controls to reduce the prevalence of *Campylobacter*.

A conflict of interest was declared by one of the panel members, the project investigator.

The reviewing panel evaluation

A general concern was raised by the panel for all BBSRC led grants, which was that it was not required for BBSRC to provide a final report to the FSA. Instead, the FSA has produced summary reports based on updates it had received and peer reviewed journal articles based on the work, which was shared with, and approved by, the BBSRC. Based on the documentation, the panel concluded that it was somewhat difficult to determine if all of the objectives on this grant (FS231081) had been met. However it was possible to see that some of the findings matched several of the objectives. The project had been met with some delays, specifically on the modelling work. It was commented that not every strain of Avian Pathogenic Escherichia coli (APEC) and *Campylobacter* interact in the same way. Chicken type is also a very important factor. The project has provided some important data on colonisation in chicken and the chicken immune response by identifying different colonisation sites and the different genotypes produce different outcomes for the bird. It was noted that there had been several publications following the completion of this work, including preparation on risk factors associated with colonisation, with the latest being of the modelling work. The interest generated indicates that the outcomes could be of significant interest.

Science: 4
Value for money: 3

FS231082

Project Title: *Campylobacter* phase variation and its impact on immunity and vaccine development.

Authors: Dr Christopher Bayliss[1]
Dr Michael Jones[2]

Institutions: [1]University of Leicester
[2]University of Nottingham

Project number: FS231082 (BB/I024542/1 [Component application BB/1024712/1])

Start date: March 2012 **End date:** March 2015

Aims of project:
The study investigated practicalities of using poultry vaccination as an effective control measure against human *Campylobacter* infection. Current vaccines have variable efficacy preventing *Campylobacter*, suggesting that bacteria can evade antibodies in a process called phase variation. Results from this study provided important evidence understanding how *Campylobacter* survives poultry vaccination.

The reviewing panel evaluation
The report refers to development and validation of a GeneScan assay to follow changes in phase variable regions of the *Campylobacter* genome, increased understanding of diversity and how *Campylobacter* changes its phase variation regions during colonisation and implications for vaccine efficacy. The project addresses one of the areas in the research priorities, which is underpinning the potential for a cost effective chicken vaccine. The summary report made it difficult to assess the relative contribution towards this given the lack of detail provided. The panel felt that that this project was of high importance and will possibly yield some very useful information. There are signs that work is still being carried out however progress has been made. It appeared some important work on gene assays was being carried out. For any follow-on work, it was expected that this would be funded by research councils. Given that current situation, it was not possible to make a judgement and therefore it was agreed to not provide an overall score.

Science: Not scored (project incomplete – no final report available)
Value for money: Not scored (project incomplete – no final report available)

FS231083

Project Title: Interventions effects on *Campylobacter* populations in poultry and poultry meat.

Authors: Professor Ian Connerton[1]

Institutions: [1]University of Nottingham

Project number: FS231083 (BB/I024585/1)

Start date: February 2012 **End date:** January 2015

Aims of project:
The project sampled all stages of broiler chicken production, from farm to retail, in order to record the type and level of *Campylobacter* contamination.

The reviewing panel evaluation

It was considered a similar project to the previous grant, FS231082. This project focussed on the biosecurity-based interventions and strategies for on-farm control. According to the summary report it appeared that the main objective was complete despite the lack of information and data presented. The models appeared to be have been completed but with little detail. It is difficult to determine if the final objectives have been completed. The summary report does claim to have identified the most effective interventions to control infection on farm but there are no further details provided. There was also another publication of one study in a well-respected journal, which does point to the work being of high quality, but it is difficult to judge given the documentation available. This project may tie in closely with FS231085. Given the current situation, it was not possible to make a judgement and therefore it was agreed to not provide an overall score.

Science: Not scored (project incomplete – no final report available)
Value for money: Not scored (project incomplete – no final report available)

FS231084

Project Title: Integrating microbiology and modelling to determine the source of *Campylobacter* infection in the broiler house and develop interventions.

Authors: Dr Ken Forbes[1]
Professor Nick Sparks[2]

Institutions: [1]University of Aberdeen
[2]SRUC

Project number: FS231084 (BB/I024623/1 [Component application BB/1024577/1])

Start date: February 2012 **End date:** January 2014

Aims of project:
This research aimed to identify the sources of *Campylobacter* in broiler house chickens, and introduce effective control measures to reduce *Campylobacter* infection.

A panel member noted a conflict of interest. This member did not participate in the review of this project.

The reviewing panel evaluation

This project was not reviewed and scored in the meeting given the lack of documentation. The pre-meeting evaluation forms stated the following:

The project included dose-response modelling and transmission modelling development, to address the priority area on quantitative modelling of interventions. Although the report is very brief, it does appear as though the original objectives have been met. It was however not possible to assess the scientific quality or technical quality of the work from the report.

Science: Not scored (project incomplete – no final report available)
Value for money: Not scored (project incomplete – no final report available)

FS231085

Project Title: Dynamics of Susceptibility and Transmission of *Campylobacter jejuni* in Chickens.

Authors: Professor Duncan Maskell[1]

Institutions: [1]University of Cambridge

Project number: FS231085 (BB/I024550/1)

Start date: October 2012 **End date:** September 2014

Aims of project:
Through the use of mathematical modelling, this study aimed to improve our knowledge and understanding of the dynamics of the interactions of the *Campylobacter* bacteria with poultry. A single experiment design was used to investigate both susceptibility and transmission of *Campylobacter* at the flock level.

The reviewing panel evaluation

This project was not reviewed and scored in the meeting given the lack of documentation. The pre-meeting evaluation forms stated the following:

There were three objectives for this project but it is difficult to draw any conclusions because of a lack of information that is available. It is not possible to comment on whether any of the objectives were achieved. It appears that the main output is clarity on the main transmission route, which is indirect environment transmission. This does appear to be important work with regards to colonisation in chicken and predictive modelling of transmission within flocks.

Science: Not scored (project incomplete – no final report available)
Value for money: Not scored (project incomplete – no final report available)

FS231086

Project Title: Modelling *Campylobacter* survival and spread through poultry processing: a population genomics approach.

Authors: Dr Sam Sheppard[1]

Institutions: [1] Institute of Life Science Medical School, Swansea University

Project number: FS231086 (BB/I02464X/1)

Start date: March 2012 **End date:** March 2014

Aims of project:
This project used state-of-the-art methods to determine the entire genetic code (genome) of *Campylobacter* strains from key stages within poultry processing and those associated with human disease. This genetic information was used to examine how variation in the physical traits (phenotype) are determined by changes to the genes (genotype).

The reviewing panel evaluation

This project was not reviewed and scored in the meeting given the lack of documentation. The pre-meeting evaluation forms stated the following:

The project goes some way in understanding the genetic basis of the factors that are important for survival in the food chain. More information on this is required. Most of the objectives appear to have been met but there are no assurances of this. The project meets the priority on predictive modelling of the system, how the bacterium survives in the food chains and studies around potential interventions. From what was available to review, it does appear that a substantial amount of work was carried out.

Science: Not scored (project incomplete – no final report available)
Value for money: Not scored (project incomplete – no final report available)

A request was made for project investigators to submit reports to the FSA so these projects could be evaluated from the FSA perspective properly. An additional request was made for PIs to describe or outline any inaccuracies/uncertainties.

FS101048

Project Title: A novel bacterial defence system against antimicrobial peptides: Implications for host colonisation in the food-borne pathogen *Campylobacter jejuni*

Authors: Professor Dave Kelly[1]
Professor Mark Stevens[2]

Institutions: [1]University of Sheffield
[2]The Roslin Institute, University of Edinburgh

Project number: FS101048

Start date: October 2013 **End date:** September 2016

Aims of project:
This research aims to determine the function of each of the components of a complex novel system in *Campylobacter jejuni*, which is involved in high-level resistance to antimicrobial peptides, and to assess its contribution to colonisation in chickens.

The reviewing panel evaluation

The aim was to determine the function of each of the components of the complex RID system in strain NCTC 11168 and its contribution to colonisation of the chicken. To date, significant progress has been made on a number of objectives. The interim report available to reviewers indicated that a lot of complex chemistry, biochemistry and molecular microbiology to investigate the resistance of one C. jejuni strain to antimicrobial peptides have been performed. There appeared to be no direct role in resistance but it was likely to have an indirect link.

The work undertaken appeared to be a typical BBSRC project with a multi-system issue. It was questioned why the FSA chose to provide funding for this work given that most outcomes are unlikely to be of interest to them. The end of year report was praised and concluded to be very good.

Science: 4 (out of 5)
Value for money: 1 (out of 5)

B15015-17

Project Title: Host acute stress responses and the regulation of *C. jejuni* virulence in the avian gut

Authors: Professor Tom Humphrey[1]
Professor Julian Ketley[2]
Mr Bruce Pearson[3]

Institutions: [1]University of Bristol
[2]University of Leicester
[3]Institute of Food Research (IFR)

Project number: B15015-17

Start date: March 2006 **End date:** February 2009

Aims of project:
This research assessed the effect of noradrenaline, produced by poultry in response to production stress, on *Campylobacter jejuni* gut colonisation in broiler chickens. This was a Biotechnology and Biological Sciences Research Council/Food Standards Agency research, funded under the Government Partnership Award (GPA) scheme.

The reviewing panel evaluation

The aim was to determine how and why host stress, involving an increase in noradrenaline excretion, affects colonisation of the chicken gut by *C. jejuni*. The project team reported noradrenaline accelerated the growth characteristics of *Campylobacter* species and induced increased motility in vitro. Excellent, high quality work had been carried out, with particular reference made to how well the research team had obtained the relevant gut information. It had met its main objective and the outcomes had already appeared to have made significant impact. For these reasons, it was excellent value for money.

Science: 5 (out of 5)
Value for money: 5 (out of 5)

B15021-22

Project Title: A nitric oxide responsive network in *Campylobacter jejuni* and its role in intracellular survival

Authors: Professor Robert K Poole[1]
Dr Simon Park[2]

Institutions: [1]University of Sheffield
[2]University of Surrey

Project number: B15021-22

Start date: March 2007 **End date:** March 2010

Aims of project:
This research examined how a newly discovered nitric oxide responsive regulator contributes to protection against nitrosative stress and intracellular survival of *Campylobacter jejuni* during infection. This was a Biotechnology and Biological Sciences Research Council/Food Standards Agency research, funded under the Government Partnership Award (GPA) scheme.

The reviewing panel evaluation
The projects appeared to have met their objectives. They had served their purpose but it is difficult to see how work could be carried forward given the complications it would likely create in having it applied. The peer review report was helpful in determining that work was carried out to a good standard. It was also possible to determine that the main objectives had been achieved. These were a) to characterise the mechanism for nitric oxide detoxification by *Campylobacter* and b) determine the roles of candidate nitric oxide detectors and a nitric oxide responsive regulator (NssR) and the role of goblins (Ctb, Ctg) produced in response to nitric oxide. The panel felt that this was possibly another example of work for which the FSA should not have provided funding given that it is not central to food safety.

Science: 5 (out of 5)
Value for money: 2 (out of 5)

End of BBSRC led grants (FSA part funded) section

FS101023

Project title: A review of recently published, peer-reviewed literature, other published information and research in progress for *Campylobacter* spp. associated with chicken broiler meat. Mapping relevant *Campylobacter* research to the poultry production chain from farmer to consumer to inform a gap analysis.

Authors: M. A. Tchórzewska[1]

Institutions: [1]School of Veterinary Sciences, University of Bristol, Langford, Bristol BS40 5DU

Project number: FS101023

Start date: 1st September 2012 **End date:** 31st March 2013

Aims of project:
This review aimed to tackle a set of questions created to identify relevant studies. These questions were:

- What factors influenced the introduction of *Campylobacter* to broilers and broiler meat?
- What was the contamination or infection prevalence and what was the degree of contamination (cfu/unit of sample) of *Campylobacter* in broiler meat since the ESFA baseline?
- Had new factors been identified that influenced prevalence and or the degree of contamination?
- What factors influenced *Campylobacter* survival in the processing environment?
- What was the risk of human infection from colonised broilers on contaminated chicken meat and the broiler production chain?
- Were there any new processing risk factors and/or interventions for human *Campylobacter* infections caused by the consumption of chicken meat?
- Were there any farm worker or processing plant operative behaviours that influenced flock infection status and/or contamination levels in broiler meat?
- Were there any consumer behaviours (e.g. washing poultry carcasses in domestic kitchens) that were influenced human infections by campylobacters from poultry sources?

Abstract

A comprehensive review of the literature relating to *Campylobacter* contamination of chicken broiler meat was undertaken by EFSA in 2010 and published in 2011 and included advice relating to *Campylobacter* control in chickens. The review was updated as part of an EFSA opinion on the revision of poultry meat inspection published in June 2012. A number of knowledge gaps were identified by the original reviews. The main aim of this study was to identify *Campylobacter* research and publications and ongoing research appropriate to any stages of the UK poultry production chain that was from farm to fork and was published since the publication of the EFSA documents.

The limited budget available for this study precluded full adherence to a systematic approach with scored assessment of each paper's quality using the Oxford System. However, within the

bounds of the project an attempt was made to systematically and reproducibly search the literature.

The review of recent literature revealed that a number of the knowledge gaps originally identified by EFSA still remained. To address these gaps, it was recommended that future studies concentrated on the following areas:

- There are gaps in our knowledge of the genetics of avian hosts, the general susceptibility of non-avian hosts and the avian immunological responses to campylobacters.
- Consumer behaviour in private kitchens, schools, catering institutions, markets.
- Food disposal practices.
- Education of the public regarding the fact that home is a likely place to acquire foodborne infections. Domestic food safety should highlight the viewpoints of preparation of both food for humans and companion animals.
- Similar education and the supply of good practices for food hygiene professionals.
- The behaviours of farm staff on poultry farms, catchers (particularly) and cleaning personnel.
- Composition and function of the chicken caecal microbiome, metabolic pathway and gene expression “from hatch to slaughter age”, including modulations of this community in response to various stressors, dietary and managerial modifications/interventions.
- Survival and spread of *Campylobacter* spp. prior entering the farm. A better understanding of the mechanisms operating that enhanced the survival of surface-attached campylobacters.
- *Campylobacter* metabolism.
- Alternative methods of poultry processing.
- Innovative retail product packaging.
- Vaccine development.

The reviewing panel evaluation

The overall objective of this project had been met but there were a number of limitations. A very broad literature review had been carried out. However, there was a distinct lack of data interrogation. The review was also restricted to the UK and a few EU member states only. There was also a criticism of the lack of synthesis that had taken place on the outcomes identified. The output was rather disappointing and there appears to be little value added other than a summary of recently published information. In all, this didn’t feel like a GAP analysis. The reviewing panel recommended that the FSA should be clearer in their expectations if a similar project is commissioned in the future.

Science: 1 (out of 5)
Value for money: 2 (out of 5)

FS121014 D

Project Title: Peer review of the process used to develop the *Campylobacter* in chicken target

Authors: Mieke Uyttendale[1]

Institutions: [1]University of Ghent.

Project number: FS121014 D

Start date: March 2012 **End date: March 2013**

Aims of project:
The aim of the review was to give a critical evaluation of the published *Campylobacter* target proposed by the Food Standard Agency (FSA) of the UK. Setting of this target was in cooperation with stalk holders from the broiler meat industry. The proposed target was released in December 2010, and was informed largely by a mathematical model (simulation) in order to estimate levels of *Campylobacter* through the broiler supply chain.

The reviewing panel evaluation

The project team had completed what had been asked of them. The objectives had been addressed very well and provided an authoritative opinion on the UK risk assessment. The correct route had been taken and the reviewing panel were in agreement by endorsing the approach. A concern was raised about the basis of outputs from the model, which would have been useful if they had been made available. The project appeared to meet FSA needs, which is consistent with the need to estimate the impact of interventions in the poultry supply chain on the levels of *Campylobacter* and population exposure. It did highlight the need for more data on *Campylobacter* levels in caeca, timing of initial infection of flocks and more on intervention methods. Overall, the work was considered to be a very good and the appropriate research team was in place given that they are internationally recognised experts in the field. It also represented good value for money. Reviewers noted that the contractors did not comment on the output of the process they were peer reviewing (i.e. whether the model gave the correct estimation of the target), however, they had not been asked to do this by the FSA, but to purely peer review the process.

Science: 5 (out of 5)
Value for money: 4 (out of 5)

FS101072

Project Title: Application of whole genome sequencing to fully characterise *Campylobacter* isolates from Infectious Intestinal Disease studies (IID1 and IID2).

Authors: Craig Winstanley[1]

Institutions: [1]University of Liverpool

Project number: FS101072

Start date: January 2014 **End date:** March 2015

Aims of project:

- fully characterise UK *Campylobacter* strains associated with human campylobacteriosis by whole genome sequencing using Next Generation Sequencing (NGS) technologies
- identify markers to assist with source attribution by integration of the data from IID study strains with published data obtained from non-human sources and genome data being derived from current funded projects at Liverpool.

Campylobacter is the most common cause of acute bacterial gastroenteritis worldwide. In the UK alone it causes an estimated 500,000 infections each year. There have been two large studies of Infectious Intestinal Disease in the UK community (IID1 in the mid 1990s and IID2 in 2008-2009). *Campylobacter* was identified as the most common bacterial pathogen amongst patients presenting to primary care. Although there was little variation in the burden of illness between the two studies, the molecular epidemiology of the *Campylobacter* isolates from these studies has not been investigated. MLST and (WGS) comparative analyses can be used to understand not only the epidemiology and any variations between the two survey periods, but also the potential sources for transmission of the pathogen to humans, by comparison with isolates from the environment, wildlife and farm animals.

Abstract

Genomic DNA from all of the *Campylobacter* isolates from the IID1 and IID2 studies were isolated. All isolate genomes were then subjected to sequencing of paired-end libraries using the Illumina MiSeq platform. After this initial run, the genomes of a sub-set of isolates (approximately 25) were improved using a PacBio approach. Using this combination approach we constructed a comprehensive genome sequence data-set from the IID isolates, and these data were submitted to a publically accessible database. We also extracted and analysed MLST data from the IID isolates, in order to place the collection in the context of previous studies. Both MLST and genomic data were used to compare between the IID1 and IID2 collections.

The major outcomes of the study were:

- a comprehensive genome sequence dataset from the IID isolates, submitted to a publically accessible database
- analysis of MLST data from the IID isolates to place the collection in the context of previous studies based on MLST
- genome-wide phylogenetic analysis of the IID strains compared to others available in the wider database (and our parallel studies involving isolates from the environment, wildlife and farm animals).

In addition, we considerably enhanced the community's knowledge on what constitutes the core genome of *Campylobacter*, especially in relation to isolates associated with human infections, with the potential to link variations between strains (either in accessory genome content, or in SNP variations within the core genome) with other factors such as putative source or, potentially, clinical severity, as well as other important phenotypes, such as survival characteristics in the environment or during food processing.

Results

WGS of all available *Campylobacter* isolates from the IID1 and IID2 studies was carried out using the Illumina platform. From the 504 samples received, WGS data was obtained for 470 *Campylobacter* isolates, comprising 351 from IID1 and 119 from IID2. Of these 416 were *C. jejuni* and 46 were *C. coli*. We also obtained WGS data from five *C. upsaliensis*, one *C. fetus*, one *Arcobacter butzleri* and one *Arcobacter* spp.

Analysis of MLST data extracted from the WGS data indicated that the most common clonal complexes found amongst the IID1 and IID2 strains reflected their abundance amongst the wider *Campylobacter* population. There were no clear variations of note between the IID1 and IID2 isolate collections with respect to breakdown according to MLST clonal complex (CC). The use of Single Nucleotide Polymorphism (SNP) phylogeny to cluster *C. jejuni* genomes based on either ribosomal loci (rMLST), or larger sets of core genes, confirmed the broad distribution of IID1 and IID2 isolates amongst the wider *Campylobacter* population, but highlighted some sub-divisions within MLST-based clonal complexes, and some small clusters specific to either IID1 or IID2. SNP-based phylogenetic analysis of *C. coli* isolates from IID1 and IID2 indicated that they all cluster within Clade 3, a clade associated previously with clinical isolates and agricultural sources.

Using PacBio sequencing, a further 17 high quality reference genomes were added to the general database, eleven of which (ten *C. jejuni* and one *C. coli*) assembled as a single chromosome. From 14 high quality PacBio genomes and three previously available complete genomes, we defined a *C. jejuni* core genome of 1,261 genes.

These data provide an excellent baseline for monitoring shifts in the UK population of *Campylobacter* associated with gastrointestinal infections. By combining survey data of this nature with analyses of other isolate collections from non-human sources, it will be possible to identify changing trends and shifts in the relative importance of potential sources of transmission.

A conflict of interest was declared by the chair and one of the panel members. These members did not participate in the review of this project.

The reviewing panel evaluation

The project had achieved its objectives of obtaining a comprehensive genome sequence dataset for isolates, analysing MLST data. The reviewing panel felt that it was excellent value for money given the number of isolates used (470) and the excellent science carried out. However, only 17 out of 25 PacBio sequences were completed, which prevented the value for money score being the highest possible. It was also felt that more work could have been carried out on comparing the isolates. Overall, it did produce good baseline data which could be very useful for subsequent work in this area. On that note, work ran at a significant loss given that there was an unexpected, but necessary, charge. It was not possible for the project team to carry out an epidemiological analysis. All of the genome information obtained from this work is now available for multilocus sequence typing (MLST).

Science: 4 (out of 5)
Value for money: 4 (out of 5

FS101106

Project Title: Factors affecting variations in *Campylobacter* disease rates in Scotland.

Authors: [1]Norval Strachan

Institutions: [1]University of Aberdeen

Project number: FS101106

Start date: January 2015 **End date:** January 2018

Aims of project:
Previous work has established that there is an apparent lower incidence of reported *Campylobacter* infections in deprived populations. However this is not observed in hospitalised cases. This project investigates the origin of these differences between deprived and prosperous populations in four ways. First, investigating potential biases at three different levels of the reporting pyramid: the community level, the GP level and the reported case level. Second analysing retrospective and prospective case and hospitalisation discharge data to determine whether the reported variation in disease still occurs. Third, carrying out a case control study to identify the sources of human Campylobacteriosis and fourth performing a case-case analysis to determine differences in risk factors for deprived and less deprived (affluent) populations. This work will provide *the* scientific evidence to inform FSA policy on dealing with *Campylobacter* risk in the Scottish population.

A conflict of interest was declared by one of the panel members. This member did not participate in the review of this project.

The reviewing panel evaluation

It was not possible to establish if the project had met any of its objectives to date. It was currently one year into a three year project. A concern was raised that FSS (previously FSA Scotland) hadn't communicated with the FSA about this project. As far as it could be seen, there had been some initial ethical issues to contend with. It appeared that at the time of the review, the project was only at the stage of circulating the questionnaires. This was raised as being rather unacceptable given the amount of time already passed. Although scores couldn't be allocated, the reviewing panel were excited about the likely outcomes from this project given their potential importance. This project is core to FSA policy requirements regarding safe food.

Science: Not scored (project incomplete – project recently started)
Value for money: Not scored (project incomplete – project recently started)

FS101087

Project Title: Generating tools for the molecular epidemiology of *Campylobacter coli* by next generation genome sequencing

Authors: van Vliet AHM [1], Pearson BM [1], Williams NJ [2], Pascoe B [3], Meric G [3], Ashton P [4], Jenkins C [4], Sheppard SK [3], Crossman LC [5,6], Wain J [7]

Institutions 1. Institute of Food Research, Norwich; 2. University of Liverpool; 3. University of Swansea; 4. Public Health England, Colindale; 5. SequenceAnalysis.co.uk, Norwich; 6. The Genome Analysis Centre, Norwich; 7. University of East Anglia, Norwich.

Project number: FS101087

Start date: 1st october 2013 **End date:** 31st october 2015

Aims of project:

- To determine the genome sequence of *Campylobacter coli* isolates from different sources
- To define the genetic variation within the core and accessory regions of the genome as defined by the selected series of *Campylobacter coli* isolates and by comparison with *C. jejuni* data.
- To compare the newly generated *Campylobacter coli* genomic information with existing typing methods
- To support development of a typing scheme specific for *Campylobacter coli* using the information obtained from the genomes.

Abstract

The foodborne bacterial pathogen *Campylobacter* is the most common cause of bacterial infectious intestinal disease in the UK, with an estimated 281,000 infections annually in the UK, and between two and 20 million cases annually in the European Union. Approximately 10-15% of these infections are caused by *Campylobacter coli* whilst the majority is caused by *C. jejuni*. Despite the large number of cases associated with *C. coli*, very little is known about its environmental reservoirs, transmission routes or mechanisms of pathogenicity. Important differences exist between *C. coli* and *C. jejuni*: different infection sources; different antibiotic resistance profiles and different genetics, and yet the evidence base for the detailed investigation of *C. coli* remains weak. It has been shown by multilocus sequence typing (MLST) and comparative genomics that *C. coli* is clearly distinct genetically from *C. jejuni*, and is divided into distinct lineages representing agricultural isolates and environmental (riparian) isolates. What is lacking for *C. coli* is sufficient resolution to allow the development of typing tools for rapid diagnostic and epidemiological investigation. With the recent developments in nucleic acid sequencing technologies it is now feasible to sequence large numbers of isolates to identify diagnostic markers and use these for future epidemiological purposes.

Results: In this project we have investigated the genetic diversity of *C. coli* using genome sequencing of 497 *C. coli* isolates from diverse sources including: animal, food, environment and human clinical cases. After assembly, genome sequences were annotated, analysed, compared and have been made publicly available. These analyses have shown that *C. coli* has a population structure comprising of 5 clearly distinct lineages, either associated with agricultural sources or environmental sources, with clinical isolates primarily grouping with the agricultural isolates. Genome coverage simulations were used to assess the coverage threshold required for the use of genome sequencing as a rapid, accurate and cost-effective

tool for *Campylobacter* epidemiology. Lineage-specific genes have been identified and tested using other publicly available datasets. Furthermore, complete genome sequences have been determined for the environmental lineages, supporting mapping-based analyses.

Conclusions: *C. coli* has a very distinct population structure, with clear separation of lineages. The genome sequences and analyses provided by this project will support future studies into the biological differences between these lineages and their significance in human disease, and development of epidemiological tools for *C. coli*.

The reviewing panel evaluation

This project has advanced our understanding of the ecology of *C. coli* (it is perhaps less complex than *C. jejuni*). From the results, it does show strongly that the environment may be an important source for human infections with *C. coli*. The project as a whole portrayed good science and involved some good sequencing. Reviewers felt that in general sequencing of strains should be used from a wide variety of sources (domestic as well as agricultural) and that in this case, more than only one pig isolate should have been sequenced, which was surprising. There was also no prospective strain collecting. The main objectives appear to have been met, and work was carried out to a high standard. There were recommendations for any subsequent genome sequencing to include a broad enough population to ensure comparisons can be made between a wide range of sources.

Science: 4 (out of 5)
Value for money: 3 (out of 5)

Annex C. *Campylobacter* research projects included in the current review

Project Code	Project Title	Start Date	End Date	Total Cost (£)
FS241049 A (M01060)	An appraisal of the efficacy and feasibility of rapid on-farm test methods for the detection of *Campylobacter*	Jan 2011	Mar 2014	305,225
FS241049 B (M01061)	Development and field evaluation of a lateral flow test for on-site detection of *Campylobacter* in poultry	Mar 2011	Mar 2014	84,000
FS101114	Small project with RVC Control data for model farms (follow-on to project FS241049 B8)-****Epidemiological analysis of *Campylobacter* data generated in an industry biosecurity project****	Jan 2014	Apr 2015	8,182
FS101123	*Campylobacter* on-farm testing of Independent broiler farms	oct 2014	Jan 2016	98,760
FS241018	Continued development of slaughterhouse hygiene tool, including extending to use on farm (Agency's slaughterhouse hygiene tool	Apr 2012	Mar 2015	312,738
FS121014 A (M01058)	Evaluate the use and cost effectiveness of steam treatment and other nine (e.g. organic acids) in the medium term	Feb 2011	Dec 2013	385,526
FS121014 B (M01059)	Collaborative research with industry – lactic acid novel interventions	Feb 2011	Feb 2013	194,392

Project Code	Project Title	Start Date	End Date	Total Cost (£)
FS241051 A	Monitoring *Campylobacter* in broiler slaughterhouses (Part A includes M01056, *Campylobacter* Proficiency testing (Feb 2011 – 2015)	Jul 2011	Mar 2016	233,560
FS241051 B	Monitoring programme for *Campylobacter* in broiler flocks and broiler carcases in the UK (with data collection, speciation and typing of isolates during the years (2012 to 2015)) – new project FS101126 is the 2016 monitoring programme for 1 year	Nov 2011	Jun 2016	1,327,226.30
FS241063 (M01055)	Investigation into changes of *Campylobacter* numbers on broiler carcasses during and following processing	Apr 2011	Mar 2014	363,560
FS990010 (M01039)	Reducing *Campylobacter* cross-contamination during poultry processing	Jul 2005	Mar 2009	71,764
FS121014 E	CampyTool Project: Comparison of the UK Slaughterhouse hygiene assessment tool and Diagnostic *Campylobacter* Tool	Feb 2013	Jul 2014	145,155
FS101038 (previously FS121014 F)	Investigation of the efficacy, practicality and cost effectiveness of modified atmosphere packaging on *Campylobacter* numbers on raw chicken intended for retail (MAP Project)	Feb 2013	Mar 2015	205,560

Project Code	Project Title	Start Date	End Date	Total Cost (£)
FS241044	A UK wide microbiological survey of *Campylobacter* contamination in fresh whole chilled chickens at retail sale	Feb 2014	Jul 2015	500,000
FS121014 C	Maintaining sentinel surveillance for human Campylobacteriosis in Oxfordshire: monitoring the impact of poultry industry interventions on the burden of human disease	Aug 2011	Aug 2014	263,354
FS101062	Controlling *Campylobacter* during the manufacture of chicken liver pate	oct 2013	Mar 2014	20,000
FS241040	Development of accurate predictive models for the assessment of the survival of *Campylobacter jejuni* and C. coli under food-relevant conditions	Apr 2011	Jan 2015	494,242
FS101042	Sources, seasonality, transmission and control: *Campylobacter* and human behaviours in a changing environment (AKA ENIGMA)	Jan 2012	Dec 2016	330,000
FS421003	Employing source attribution and molecular epidemiology to measure the impact of interventions on human Campylobacteriosis in Scotland	Nov 2011	Jan 2016	754,875

Project Code	Project Title	Start Date	End Date	Total Cost (£)
FS231081-86 (BBSRC led Projects)	**BBSRC led '6 projects'**	Aug 2011	Various	965,723.15. contribution for all six projects
FS231081 (BB/I024674/1)	How production systems, bird welfare and endemic disease affect the susceptibility of chickens to *Campylobacter*			
FS231082 BB/I024542/1 [Component application BB/1024712/1])	*Campylobacter* phase variation and its impact on immunity and vaccine development			
FS231083 (BB/I024585/1)	Interventions effects on *Campylobacter* populations in poultry and poultry meat			
FS231084 (BB/I024623/1 [Component application BB/1024577/1])	Integrating microbiology and modelling to determine the source of *Campylobacter* infection in the broiler house and develop interventions			
FS231085 FS231085 (BB/I024550/1)	Dynamics of susceptibility and transmission of *Campylobacter jejuni* in chickens			
FS231086 - FS231086 (BB/I02464X/1)	Modelling *Campylobacter* survival and spread through poultry processing: a population genomics approach.			

Project Code	Project Title	Start Date	End Date	Total Cost (£)
FS121014 D	Peer review of the process used to develop the *Campylobacter* in chicken target	Mar 2012	Mar 2013	10,000
FS101072	Application of whole genome sequencing to fully characterise *Campylobacter* isolates from Infectious Intestinal Disease studies (IID1 and IID2)	Jan 2014	Mar 2015	125,700
FS101087	Generating tools for the molecular epidemiology of *Campylobacter* coli by next generation sequencing. Part of the Strategic Challenge rather than the *Campylobacter* Research Programme	Aug 2013	Jul 2015	102,951
FS101106	Factors affecting variations in *Camplylobacter* disease rates in Scotland	Jan 2015	Jan 2018	300,000
FS101025	To assess the impact of freezing on *Campylobacter* on chicken livers	oct 2012	Mar 2013	10,000

Project Code	Project Title	Start Date	End Date	Total Cost (£)
FS101048 (BBSRC led project - BB/K005510/1 & BB/K005642/1)	A novel bacterial defence system against antimicrobial peptides: Implications for host colonisation in the food-borne pathogen *Campylobacter jejuni*	May 13	Apr 16	111,000
FS101023	Mapping relevant *Campylobacter* research to the poultry production chain from farmer to consumer to inform a gap analysis	Sep 12	Mar 13	£10,000

Annex D. Publications to have arisen following FSA-funded research

FS101042

Jones, A.K., Rigby, D., Burton, M., Millman, C., Williams, N.J., Jones, T.R., Wigley, P., O'Brien, S.J., Cross P., (2016) **Restaurant Cooking Trends and Increased Risk for *Campylobacter* Emerging Infectious Disease**. *Infection* Volume 22, Number 7. July 2016

Bronowski, C., James, C E., Winstanley, C., (2014) **Role of environmental survival in transmission of *Campylobacter* jejuni.** FEMS Microbiol Lett 356 8–19

FS101062

Hutchison M, Richardson I., (2015) **A Method for the Preparation of Chicken Liver Pâté that Reliably Destroys Campylobacters,** Int. J. Environ. Res. Public Health **2015**, 12, 4652-4669.

FS231081

Humphrey, S., (2014) ***Campylobacter jejuni* is not merely a commensal in commercial broiler chickens and affects bird welfare**, mBio, vol. 5, no. 4, pp. 01364-14.

Chaloner, G., (2014) **Dynamics of dual infection with *Campylobacter jejuni* strains in chickens reveals distinct strain-to-strain variation in infection ecology**, Applied and environmental microbiology, vol. 80, no. 20, pp. 6366-72.

FS231083

Newell, D.G., (2011) **Biosecurity-based interventions and strategies to reduce *Campylobacter* spp. on poultry farms**, Applied and environmental microbiology, vol. 77, no. 24, pp. 8605-14

FS231084

Ovidiu, R. (2013) **An integrated model to estimate the source of *Campylobacter* infection in broiler houses**., Journal of Medical Microbiology, Conference Proceedings Abstract; Forbes K 2013, 'The 17th International Workshop on Campylobacter, Helicobacter and Related Organisms.', Journal of Medical Microbiology.

FS231085

Holt J.P. (2012), **Identification of Cj1051c as a major determinant for the restriction barrier of *Campylobacter jejuni* strain** NCTC11168.', Applied and environmental microbiology, vol. 78, no. 22, pp. 7841-8; Djamila Moulay 2013, 'A mechanical hypothesis for the lag phase of Broiler colonization with *Campylobacter* jejuni', Conference Proceedings Abstract

FS231086

Sheppard SK, Cheng L, Méric G, de Haan CPA, Llarena A-K, Marttinen P, Vidal A, Ridley A, Clifton- Hadley F, Connor TR, Strachan NJC, Forbes K, Colles FM, Jolley KA, Bentley SD, Maiden MCJ, Hänninen M-L, Parkhill J, Hanage WP, Corander J (2014) **Cryptic ecology among host generalist *Campylobacter jejuni* in domestic animals**. **Mol Ecol**. 2014 (in press)

Meric G, Yahara K, Mageiros L, Pascoe B, Maiden MCJ, Jolley KA, Sheppard SK. (2014) **A reference pangenome approach to comparative bacterial genomics: identification of novel epidemiological markers in pathogenic *Campylobacter***. **Plos One**. 2014

Yahara K, Didelot X, Ansari MA, Sheppard SK and Falush D. **Efficient inference of recombination hot regions in bacterial genomes** (2014). **Mol Biol Evol**. 2014 (in press)

.Torralbo A, Borge C, Allepuz A, García-Bocanegra I, Sheppard SK, Perea A, Carbonero A (2014) **Prevalence and risk factors of *Campylobacter* infection in broiler flocks from southern Spain**. **Prev Vet Med**. 2014 Jan 30. pii: S0167-5877(14)00034-8. doi: 10.1016/j.prevetmed.2014.01.019

Sheppard SK, Méric G.(eds.) (2014) ***Campylobacter* Ecology and Evolution**, Caister Academic Press, April 2014, c.350 pages ISBN: 978-1-908230-36-2.

Brisse S, Brehony C, Conceição T, Cubero M, Glasner C, Le Gouil M, Renvoisé A, Sheppard S, Weinert L. (2014) **Microbial molecular markers and epidemiological surveillance in the era of high throughput sequencing: an update from the IMMEM-10 Conference**. **Res Microbiol.** 2014 (in press)

Strachan NJ, Rotariu O, Macrae M, Sheppard SK, Smith-Palmer A, Cowden J, Maiden MC, Forbes KJ. (2013) **Operationalising factors that explain the emergence of infectious diseases: a case study of the human Campylobacteriosis epidemic**. **PLoS One**. 2013 Nov 21;8(11):e79331. doi: 10.1371/journal.pone.0079331.

Wimalarathna HML, Richardson JF, Lawson AJ, Elson R, Meldrum R, Little CL, Maiden MCJ, McCarthy ND, Sheppard SK (2013) **Widespread acquisition of antimicrobial resistance among *Campylobacter* isolates from UK retail poultry and evidence for clonal expansion of resistant lineages**. **BMC Microbiol**. 2013 Jul 15;13:160.

Sheppard SK, Didelot X, Meric G, Torralbo A,, Jolley KA, Kelly DJ, Bentley SD, Maiden MCJ, Parkhill J, Falush D. (2013) **Genome-wide association study identifies vitamin B5 biosynthesis as a host specificity factor in *Campylobacter***. **Proc Natl Acad Sci U S A**. 2013 Jul 16;110(29):11923-7.

Roux F, Sproston E, Rotariu O, MacRae M, Sheppard SK, Bessell P, Smith-Palmer A, Cowden J, Maiden MCJ, Forbes KJ, Strachan NJC. (2013) **Elucidating the Aetiology of Human *Campylobacter coli* Infections. PLoS One**. 2013 May 29;8(5):e64504.

Sheppard SK, Didelot X, Jolley KA, Darling AE, Pascoe B, Meric G, Kelly DJ, Cody A, Colles FM,Strachan NJ, Ogden ID, Forbes K, French NP, Carter P, Miller WG, McCarthy ND, Owen R, Litrup E, Egholm M, Affourtit JP, Bentley SD, Parkhill J, Maiden MC, Falush D. (2013) **Progressive genome-wide introgression in agricultural *Campylobacter coli***. **Mol Ecol**. 2013 Feb;22(4):1051-64.

Read DS, Woodcock DJ, Strachan NJ, Forbes KJ, Colles FM, Maiden MC, Clifton-Hadley F, Ridley A, Vidal A, Rodgers J, Whiteley AS, Sheppard SK. **Evidence for phenotypic plasticity amongst multihost *Campylobacter jejuni* and *C. coli* lineages using ribosomal MLST and Raman spectroscopy**.(2013) **Appl Environ Microbiol**. 2013 Feb;79(3):965-73.

Asakura H, Brüggemann H, Sheppard SK, Ekawa T, Meyer TF, Yamamoto S, Igimi S. (2012) **Molecular Evidence for the Thriving of *Campylobacter jejuni* ST-4526 in Japan**. **PLoS One**. 2012;7(11):e48394.

Strachan NJ, Rotariu O, Smith-Palmer A, Cowden J, Sheppard SK, O'Brien SJ, Maiden MC, Macrae M, Bessell PR, Matthews L, Reid SW, Innocent GT, Ogden ID, Forbes KJ. (2013) **Identifying the seasonal origins of human Campylobacteriosis**. **Epidemiol Infect**. 2013 Jun;141(6):1267-75.

Nichols GL, Richardson JF, Sheppard SK, Lane C, Sarran C. (2012) ***Campylobacter* epidemiology: a descriptive study reviewing 1 million cases in England and Wales between 1989 and 2011**. **BMJ Open**. 2012 Jul 12;2(4).

Annex E. Examples of the programme's impact on FSA policy

Finished or ongoing projects

FS101025 *Freezing as an intervention to reduce the numbers of campylobacters isolated from chicken livers* (refer to page 43 for the full review). The project came to the conclusion that freezing chicken livers can help to reduce *Campylobacter* numbers and that freezing for a second period will reduce them further but that the method will not eliminate them completely. This has offered a practical solution/working intervention to industry, in particular food business operators and caterers that regularly use chicken livers.
http://www.food.gov.uk/science/research/foodborneillness/b14programme/b14projlist/fs101025

FS101062 *Controlling Campylobacter during the manufacture of chicken liver pâté* (refer to page 45 for the full review). The project successfully produced a safe method for the production of liver pâté which food business operators (FBOs) can use when preparing this high risk food. Environmental Health Officers (EHOs) within Local Authorities had eagerly awaited its publication given that FBOs could adhere to the recommendations and the LAs could help to raise its awareness with FBOs that prepared chicken liver pâté. It provides EHO's with a useful guide when checking FBO compliance.
https://www.food.gov.uk/science/research/foodborneillness/b14programme/b14projlist/fs101062

FS241044 *A UK wide microbiological survey of Campylobacter contamination in fresh whole chilled chickens at retail sale* was well documented in the news as it led to the publication of chicken *Campylobacter* levels broken down by major retailers (refer to page 40 for the full review). Tackling *Campylobacter* levels in chicken is the FSA's top priority in the fight against food poisoning and we wanted consumers to have the clearest possible information on the food they buy. We wanted to set a clear expectation for poultry producers and retailers to take action to reduce levels of *Campylobacter* in chicken. As a result, this first survey was a key lever in influencing retailers and industry to trial new initiatives and technologies in an effort to reduce the level of contamination on fresh whole chilled chickens.
http://www.food.gov.uk/science/research/foodborneillness/b15programme/b15projects/fs241044a

FS121014 D *Peer review of the process used to develop the Campylobacter in chicken Target* was undertaken in 2012 (refer to page 65 for the full review). Since 2009 we have been working with industry to tackle Campylobacter in poultry through a Joint Working Group (JWG) (now known as the ACT Board). The original JWG agreed a target to reduce the levels of *Campylobacter* in UK-produced fresh chicken and developed an Action Plan to deliver the target. The target was considered achievable for the reduction of *Campylobacter* contamination of UK-produced chickens. It was informed largely by a mathematical model. The aim was to reduce the percentage of the most heavily contaminated chickens, with more than 1000 colony forming units per gram of chicken skin (cfu/g) at the end of the slaughter process, from 27% in 2008 to 19% by 2013, and to 10% by 2015. A review was carried out (FS121014 D) to give a critical evaluation of the published *Campylobacter* target, which was an early example

of major FSA policy setting associated with *Campylobacter*. It was concluded that the model had good validity and sound robustness.
https://www.food.gov.uk/science/research/foodborneillness/b14programme/b14projlist/fs121014d

FS241051 B *Monitoring Programme for Campylobacter in Broiler Flocks and Broiler Carcases in the UK* provided the results which could be used to pinpoint progress towards the joint industry target (refer to page 30 for the full review). The original monitoring programme was run by APHA and included the monitoring of slaughter batches and carcasses. It provided baseline *Campylobacter* data to feed into the risk assessment models. This helped to determine how well it was meeting the necessary criteria as outlined in the joint industry target.
https://www.food.gov.uk/science/research/foodborneillness/b15programme/b15projects/fs241051B

FS241018 *Continued development of the Agency's slaughterhouse hygiene tool, including extension for use on-farm* was carried out to make improvements on the already existing system (refer to page 22 for the full review). A number of modifications improved the experience of processors and allowed them to carry out additional functions, one of which was to record/monitor their results and make comparisons with the National average. This allowed them to make any potential improvements, which again all fed into the joint industry target.

FS241063 *Investigation into changes of Campylobacter numbers on broiler carcasses during and following processing* has been beneficial in helping to improve understanding of critical points in the slaughterhouse environment (refer to page 32 for the full review). The recommendations allow the individual slaughterhouses to focus on specific points which appear to be high risk for increasing *Campylobacter* levels on carcasses. They are then able to assess what improvements could be made to reduce or to better control this risk. The findings have been incorporated into a series of posters and have been used to advise on-site FVO's. This was part of an FSA initiative entitled the '*Campylobacter* Abattoir campaign' which involved more than 70 meat hygiene inspectors and official veterinarians working closely with poultry plant operators throughout the UK. It used scientific evidence to advise them on practical ways in which processing practices could be improved. This covered each stage of processing – from lairage, scalding and plucking through to evisceration, washing and chilling.
https://www.food.gov.uk/science/research/foodborneillness/b15programme/b15projects/fs241063

FS121014 A *Efficacy and Practicality of Rapid Surface Cooling, Electrolysed Water, Ultra-Violet Radiation, Steam, and Hot Water for Campylobacter Reduction &* ***FS121014 B*** *Efficacy and Practicality of Lactic Acid Solutions, Ozonated Water, and Cold Plasma for Campylobacter Reduction* were two intervention-based projects undertaken by Campden BRI which helped to inform discussions at the original Campylobacter Joint Working Group (JWG) (refer to pages 24 - 26 for the full review). They also provided useful information for industry, allowing permitted interventions to be trialled in order to reduce *Campylobacter* levels on the carcass.
https://www.food.gov.uk/science/research/foodborneillness/b15programme/b15projects/fs121014a

https://www.food.gov.uk/science/research/foodborneillness/b15programme/b15projects/fs121024B

FS101042 *Sources, seasonality, transmission and control: Campylobacter and human behaviour in a changing environment (ENIGMA)* a long-term, 5 year project which is due to finish at the end of 2016 (refer to page 50 for the review to date). It has already produced outcomes which have improved policy understanding of *Campylobacter*, specifically how it relates to the wider environment outside of poultry contamination. The project is also significantly contributing to the development of the new foodborne disease strategy 2017-2022. It has also highlighted the need to work across government and wider, especially for new research as this will continue to encourage interdisciplinary science.
https://www.food.gov.uk/science/research/foodborneillness/b14programme/b14projlist/fs101042