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# FS516002: Extension to FS145003 (Trial of visual inspection of fattening pigs from non-controlled housing conditions) - additional molecular diagnostics.

Final report for:

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Scotland's Rural College - SRUC was formed on 1 October 2012 by the merger of Barony, Elmwood and Oatridge Colleges and SAC (Scottish Agricultural College).

# EXECUTIVE SUMMARY

## FINAL REPORT FOR PROJECT FS516002

In previous work (Project FS145003) – "A Trial of visual inspection of fattening pigs from non-controlled housing conditions" – the frequency and types of conditions that could be identified at slaughter by two different meat inspection methods in carcases from fattening pigs, reared outdoors, in the UK, were investigated. We wanted to see if there was a difference between the two methods that might lead to a change in risk to human health, animal health, or animal welfare, if the inspection method was changed for pigs reared outdoors. The traditional method involved visual inspection with additional handling and incisions to investigate parts of the carcass and offal (the internal organs and entrails) that cannot be easily observed from the outside. With the visual-only method no handling of the carcass and offal was allowed. We also took carcass swabs after each inspection method to see if we could grow particular bacteria. This was to try to assess if there would be a reduction in food-borne risk due to reduced microbial carcass contamination when the carcases were handled less.

The field study involved five separate weeks of work in one abattoir in the East of England from November 2011 until April 2012. This abattoir was used because it had sufficient throughput and sufficient space to be able to run the study. For *Salmonella* spp. isolation, 800 swabs were taken (400 after the traditional inspection point and 400 after the visual-only inspection point) during the four weeks of the trial from January 2012 to April 2012. For *Yersinia* spp. isolation, a total of 759 swabs were tested in the whole study; 160 in November 2011 and 599 in the four weeks from January until April 2012 (379 after traditional inspection and 380 after visual-only inspection).

Traditional microbiological techniques isolated 12 *Yersinia* and no *Salmonella*. No statistical difference was found in the proportion of carcases contaminated with *Yersinia* spp. We were looking for *Yersinia* spp. and *Salmonella* spp. because they are known to be carried by pigs and sometimes they can cause disease in humans. The low numbers of bacteria found (i.e. the microbiological outcomes) were not totally unexpected, as it was acknowledged that the study abattoir had an extremely high level of hygiene. This was also evident from the low levels of total aerobic and coliform bacterial counts that we obtained.

To check our findings, we arranged this study (FS516002) to carry out additional molecular testing on samples that had been stored from the original study. We used polymerase chain reaction (PCR) tests to see if they could detect deoxyribonucleic acid (DNA) specific for each of the bacteria, *Salmonella* and *Yersinia* spp. If the specific DNA can be detected it indicates the presence of that bacteria in the sample tested. It does not indicate whether the bacteria are viable (alive), and could be cultured by traditional methods, or dead. We also wanted to use some additional techniques to find out more about the species of *Yersinia* that we had found.

This additional molecular diagnostic testing:

- did not identify any further samples that were positive for *Yersinia*
- did not identify any samples that were positive for *Salmonella*

- gave us further information on the biotypes and serotypes of *Yersinia* spp. present and confirmed biochemically that the *Yersinia enterocolitica* and *Yersinia frederiksenii* recovered by culture from the original carcass swab samples were *Yersinia*.

We confirmed, therefore, that the results from the original microbial investigations were valid. They reflect the microbiological status of the pigs sampled on the line and the good hygiene practices in place in this abattoir.

From this additional molecular diagnostics study we can make the following recommendations:

1. For any study that involves identification of bacterial isolates the questions that are to be asked within the study should be clearly identified first. i.e. Why are we doing this? What are we looking for? This will lead to the identification of the most appropriate diagnostic methods to use.

2. When identification of bacteria is required, then ISO standard PCR based testing for the specific bacteria could be used on enrichment samples first, followed by culture only of those samples that give a positive signal. This could optimise resource use. However, this approach would need thorough validation via an appropriately designed trial.

# FINAL REPORT FOR FS516002: EXTENSION TO FS145003 'TRIAL OF VISUAL INSPECTION OF FATTENING PIGS FROM NON-CONTROLLED HOUSING CONDITIONS': ADDITIONAL MOLECULAR DIAGNOSTICS


## ACKNOWLEDGEMENTS

This project (FS516002) and the preceding project (FS145003) were funded by the Food Standards Agency (FSA).

This project could have not been performed without the help of all the participants in the previous project team.

We also thank Dr Claire Jenkins, Head of Gastrointestinal Infections Reference Unit (GIRU), Public Health England (PHE), and her team; especially Ms Marie Chattaway, Mrs Dawn Hedges and Dr Andy Lawson for their help, time and use of facilities for biochemical identification and serotyping of *Yersinia* isolates plus real time polymerase chain reaction (PCR) testing for *Salmonella* deoxyribonucleic acid (DNA).


## THE PROJECT TEAM

The project team was led and managed by Dr Sue C. Tongue, Epidemiology Research Unit (ERU), SRUC. She had overall responsibility for the project including the budget, reporting, finalising all reports and FSA liaison.

Judith Evans (ERU, SRUC) was responsible for the laboratory work and its organisation, liaison with PHE, management of the laboratory data and drafted this report.

Both project team members contributed to the Final Report.

## Version Control

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## FINAL REPORT FOR FS145003

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# FINAL REPORT FOR PROJECT FS516002

## BACKGROUND

In the original project, FS145003 – "A trial of visual inspection of fattening pigs from non-controlled housing conditions" – the frequency and types of conditions that could be identified at slaughter by two different meat inspection methods in carcases from pigs, reared outdoors, in the United Kingdom (UK), were investigated. The traditional inspection method involved visual inspection with additional handling and incisions to investigate parts of the carcass and offal (the internal organs and entrails) that cannot be easily observed from the outside. With the visual-only inspection method no handling of the carcass and offal was allowed. We also took carcass swabs after each inspection method to see if we could grow particular bacteria. This was to try to assess if there would be a reduction in food-borne risk due to reduced microbial carcass contamination when the carcases were handled less.

The carcases we looked at were from pigs reared in outdoor management systems entirely from weaning to submission for slaughter. As far as we are aware, all such pigs in the UK are also born in outdoor management systems. We looked at these pigs because there was a perception that, as the disease status of pigs raised in indoor, controlled, integrated systems can be more readily quantified and will be more uniform than those from outdoor, non-controlled, non-integrated systems, they (outdoor reared pigs) may pose a more variable risk, if inspection systems were to be changed. Fattening pigs raised entirely outdoors, from weaning to slaughter, would be more likely to be different to pigs raised totally indoors, than fattening pigs raised partially outdoors and partially indoors; therefore they would potentially present the greatest risk. Although a qualitative risk assessment (Hill *et al*., 2011) of the comparative risks to public and animal health from visual inspection of indoor and outdoor pigs concluded that the risk was negligible for all pigs.

The field study involved five separate weeks of work in one abattoir in the East of England during the period from November 2011 until April 2012. There was only one study premise due to difficulties in identifying abattoirs that had a sufficient throughput of fattening pigs that met the definition of being from non-controlled (outdoor) housing conditions and had space to safely set up the two inspection points.

For *Salmonella* spp. isolation, 800 swabs were taken (400 after the traditional inspection point and 400 after the visual-only inspection point) during the four weeks of the trial from January 2012 to April 2012. For *Yersinia* spp. culture, a total of 759 swabs were tested; 160 in November 2011, and 599 between January and April 2012; 379 after traditional inspection and 380 after visual-only inspection. Traditional microbiological techniques isolated 12 *Yersinia* and no *Salmonella*.

The microbiological outcomes of the primary study were not totally unexpected, as it was acknowledged that the study abattoir had an extremely high level of hygiene. This was evident from the low levels of total aerobic and coliform bacterial counts. To corroborate our findings, we arranged (FS516002) to carry out molecular testing on samples that had been stored from the original study to detect DNA specific for *Salmonella* and *Yersinia* spp. If the specific DNA can be detected it indicates the presence of that bacteria in the sample tested. It does not indicate whether the bacteria are viable (alive), and could be cultured by

traditional techniques, or dead. We also wanted to find out more about which species of *Yersinia* we had found.

We were looking for *Yersinia* spp. and *Salmonella* spp. because they are known to be carried by pigs and they can cause disease in humans. In the human gastrointestinal tract *Yersinia (Y.) enterocolitica* can cause a range of symptoms such as bloody diarrhoea, acute enteritis, terminal ileitis, and mesenteric lymphadenitis. Sequelae including reactive arthritis and erythema nodosum occur in some cases (Rosner *et al*.). In 2010 the UK reported confirmed yersiniosis in humans at a rate of 0.09 per 100,000 head of population (Anon, 2012), although *Y. enterocolitica* can be isolated at similar rates from the faeces of symptomatic and asymptomatic individuals (Food Standards Agency).

Pigs are considered a primary reservoir of strains that are pathogenic for humans (Anon, 2010, Laukkanen *et al*. and Virtanen *et al*.) and rates of isolation of *Yersinia* from pig carcass swabs ranging from 0-5% using culture methods have been reported (Gürtler *et al*., Lindblat *et al*., and Nesbakken *et al*.). *Yersinia* can easily enter the food chain by contamination of carcases during slaughter and subsequent contamination of meat and meat products. Biotyping distinguishes *Yersinia* associated with human disease (notably 1B and biotypes 2-5) from biotype 1A, which are generally considered non-pathogenic for humans (Bottone).

*Salmonella* bacteria are widespread in human and animal populations. There are a large (approximately 2500 strains) number of *Salmonella* serotypes that cause food poisoning, typhoid and paratyphoid fevers in humans. Clinical signs of food poisoning include diarrhoea, stomach cramps and sometimes vomiting and fever, which usually last for four to seven days and is self-limiting i.e. it clears up without treatment. However, it can be more serious and cause dehydration in young children, the elderly and people whose immune systems are not working properly.

Pigs can carry *Salmonella* in their gastrointestinal tracts, and the organism has been recovered from a range of sites, including surface of the carcass, faeces and the mesenteric lymph node (Botteldoorn *et al*.). Infection is commonly either asymptomatic or subclinical (Anon, 2010) and the organism can enter the food chain through many routes including via contaminated pig meat. From October 2006 to September 2007 the UK prevalence of *Salmonella* recovered from pig carcases was 13.5% (Anon, 2008). Published results of abattoir studies on pig carcass and faeces report *Salmonella* recovery rates of 23-34% (Botteldoorn *et al.,* Milnes *et al*.). Work by Davies, *et al*., (2004) recovered Salmonella in 23% of pig caecal contents and 5.3% from carcass swabs. A subsequent study identified Salmonella in 23.4% of caecal contents (Milnes, *et al*., 2007).

## THE PROJECT

The aim of the project was two-fold. In FS145003, the primary study, we isolated 12 *Yersinia* spp. on culture of 759 carcass swabs and our first aim was to use more intensive biochemical procedures to confirm the identity of these organisms. Our second aim was to screen samples that had been stored at -80°C since the original sample processing, using DNA based tests, in order to investigate if we could substantiate the results from the traditional microbiological work.

## Project structure

The five objectives were:

Objective 1: Identify *Yersinia* isolated in FS145003 (Biotype and Serotype)

Objective 2: Determine the presence or absence of *Yersinia* and *Salmonella* DNA in frozen samples, archived from FS145003, using PCR testing and analysis

Objective 3: Compare the molecular testing results with the traditional culture results from FS145003

Objective 4: Report on the outcomes

Objective 5: Prepare a paper for submission for peer-reviewed publication

## Methods

### Microbiological investigations in FS145003

Sponge swabs collected from the surface of pig carcases in the study premises were processed in the laboratory 24 hours after collection in the abattoir. Culture for *Salmonella* and *Yersinia* were carried out on slightly different sample subsets.

All 160 carcass swabs collected in November 2011 were cultured only for *Yersinia* spp. Between January and April 2012 a further 800 carcass swabs were collected. Of these, 599 were cultured for *Yersinia* spp., whereas all 800 were cultured for *Salmonella* spp.

***Yersinia* spp**. isolation was carried out using the following methods:

BS EN ISO 10273:2003 (BSI, 2003)

Standard Method F33: Issue 1.0. (HPA, 2011a)

***Salmonella* spp**. isolation was carried out using the following methods:

BS EN ISO 6579:2002 (BSI, 2002) and

Standard Method F13: Issue 1(HPA, 2011)

A one millilitre aliquot from each original maximum recovery diluent (MRD), extracted from the swab sample, was retained at -80°C for the 800 carcass swabs collected between January and April 2012.

No MRD samples were retained at -80 °C for the carcass swabs collected in November 2011.

### Sample processing

***Objective 1: Identify Yersinia isolated in FS145003 (Serotype and Biotype)***

Twelve organisms had been identified as *Yersinia* during the original study using API 20E identification strips (bioMérieux SA**.** F-69280 Marcy l'Etoile, France). This is a commercial system that provides 21 biochemical tests and database support to identify bacteria. The organisms were kept on nutrient agar slopes and transported to PHE, England. Each

organism was then subcultured onto MacConkey, blood and Congo red magnesium oxalate (CMO) agars to check viability, purity and identify potentially pathogenic organisms.

OmniLog

The OmniLog® Phenotype MicroArray™ (Biolog. Inc. 21124 Cabot Blvd. Hayward, CA 94545. U.S.A.) is a commercial system providing a wide range of substrates and software support to identify bacteria.

Single colonies were picked from blood agar plates to inoculate a broth, provided by Omnilog, that was dispensed into an individual microtitre plate (also provided by Omnilog) and placed into the Omnilog incubator. Ninety four sugar and chemical substrates were used to provide a finger-print for each of the bacteria that we had identified as *Yersinia* using the API20E system. Incubation time was 22 hours and results were analysed using the Omnilog system software before being further refined using an in-house algorithm.

Traditional Biochemistry

Single colonies were picked from either the blood or CMO agar plates to inoculate a tube of peptone water which was then incubated at 28°C for a minimum of 2 hours. A single drop of peptone water was used to inoculate various tubes of substrates including glucose, cellobiose, mucate, urea, aesculin and pyrazinamidase as well as a Tween 80 agar plate. Biochemical reactions were recorded and used to identify the bacteria.

Serotyping

Screening plates were prepared containing 40µl of serum per well. An equal volume of test antigen suspension (40µl/well) was added to each well and incubated at 50°C overnight. The plates were read and results (positive = agglutination) recorded. Using fresh microtitre plates titrations, doubling dilutions in saline from 1/100 to 1/6400 of the sera that gave positive reactions, were performed; each titration had a saline (-ve) control. An equal volume of antigen suspension was added and the plates were incubated at 50°C overnight. Significant titres were re- tested using pure absorbed antisera

***Objective 2: Determine the presence or absence of Yersinia and Salmonella DNA in frozen samples, archived from FS145003, using PCR testing and analysis***

The original stored 1ml MRD samples were defrosted, boiled for ten minutes and split into two aliquots. One was returned to our -80C storage, the other transported to GIRU at PHE, London. The stored aliquot was tested with end-point PCR (Table 1), for *Yersinia* DNA; electrophoresis was carried out either in 2% gels for *Yersinia* invasin (*inv*) PCR or 2.5% gels for *Yersinia* heat stable enterotoxins *ystA* and *ystB* PCR, taking approximately 5½ hours to test 80 samples for each target. *Salmonella* specific DNA (ttr, tetrathionate reductase) was sought using real-time PCR (Table 1), taking approximately 1 hour to test 93 samples.

**Table 1: PCR probe and primer sequences used in this study**

| Primer/Probe | Sequence (5'→3') | Annealing Temperature | Reference |
|---|---|---|---|
| *Yersinia ystA*<br>Forward<br>Reverse | <br>ATCGACACCAATAACCGCTGAG<br>CCAATCACTACTGACTTCGGCT | 61°C | Thoerner 2003 |
| *Yersinia ystB*<br>Forward<br>Reverse | <br>GTACATTAGGCCAAGAGACG<br>GCAACATACCTCACAACACC | 61°C | Thoerner 2003 |
| *Yersinia Inv*<br>Forward<br>Reverse | <br>CGGTACGGCTCAAGTTCCTCTG<br>CCGTTCTCCAATGTACGTATCC | 61°C | Thoerner 2003 |
| *Salmonella ttr*<br>Forward<br>Reverse<br>Probe | <br>CTCACCAGGAGATTACAACATGG<br>AGCTCAGACCAAAAGTGACCATC<br>CACCGACGGCGAGACCGACTTT | 60°C | Malorny 2004 |

## Results

***Objective 1: Identify Yersinia isolated in FS145003 (Biotype and serotype)***

In the original study, twelve *Yersinia* spp. were isolated from culture of 759 carcass swab. Eleven of these were available for further study. Five were identified as *Yersinia enterocolitica*, six as *Yersinia frederiksenii* and one failed to grow on subculture (Table 2).

**Table 2: Identification, biotype and serotype of *Yersinia* spp. confirmed at PHE.**

| Presumptive identity in FS145003 | Confirmed identity in FS516002 | Biotype | Serotype | Week of original field trial |
|---|---|---|---|---|
| *Y. enterocolitica* | *Y. enterocolitica* | 4 | O:3 | 1 |
| *Y. enterocolitica* | *Y. enterocolitica* | 3 | O:5, 27 | 1 |
| *Y. enterocolitica* | *Y. enterocolitica* | | | 1 |
| *Y. enterocolitica* | *Y. enterocolitica* | 2 | O? | 5 |
| *Y. pseudotuberculosis* | *Y. enterocolitica* | 3 | O:5, 27 | 1 |
| *Y. enterocolitica* | No growth on repeated subculture | | | 1 |
| *Y. enterocolitica* | *Y. frederiksenii* | | O4, 32 | 1 |
| *Y. enterocolitica* | *Y. frederiksenii* | | O4, 32 | 1 |
| *Y. enterocolitica* | *Y. frederiksenii* | | O4, 32 | 1 |
| *Y. enterocolitica* | *Y. frederiksenii* | | O4, 32 | 3 |
| *Y. enterocolitica* | *Y. frederiksenii* | | O4, 32 | 4 |
| *Y. frederiksenii* | *Y. frederiksenii* | | O Rough | 1 |

These results may appear to contradict one another, however, the identification systems used differ in the range of tests performed: API20E uses 21 tests, the Omnilog uses 94, and the traditional biochemistry method uses 41. It is unsurprising then, that extra testing was able to further refine our initial findings.

From the additional characterisation work carried out we found five *Y. enterocolitica,* one of these was biotype 4, serotype 3 (*Y. enterocolitica* 4/O:3), two were *Y. enterocolitica* 3/O:5,27, one was *Y. enterocolitica* 2/O:? and serology results were unavailable on the fifth.

We found six *Y. frederiksenii*, five were O:4, 32 and the remaining was O:Rough

During the original study (FS145003), 160 carcass swabs were tested for Yersinia in week 1 (the first week in November 2011); nine *Yersinia spp.* were isolated. For the rest of the trial (four weeks between 16th January 2012 and 16th March 2012) 599 carcass swabs were tested for Yersinia; a total of three *Yersinia* spp. were isolated (Table 2).

***Objectives 2 and 3: Determine the presence or absence of Yersinia and Salmonella DNA in frozen samples, archived from FS145003, using PCR testing and analysis.***

***Compare the molecular testing results with the traditional culture results from FS145003***

Two different approaches were used for PCR.

The *Yersinia* PCR used an end-point approach, that is, the PCR reaction was run to completion and amplification products were separated in an agar gel and visualised under ultra-violet (UV) light.

Detection of *Salmonella* DNA used real-time PCR which allows products of amplification to be detected as the reaction progresses and does not require post-amplification treatment.

No new samples were identified as having DNA for *Yersinia*.

No samples were identified as having *Salmonella* DNA in them. This confirms the results of our traditional isolation methods.

***Outcomes from the previous analysis in FS145003***

Full details of the statistical analysis for the original field trial can be found in Annex 3 of the Final report for FS145003. Here we reiterate some findings that relate to the *Yersinia* isolates and the hygiene process indicators, total aerobic count (TAC) and *Enterobacteriaceae* counts that were done using the carcass swabs collected during the field trial.

There was no recording of the batch (farm of origin of the pigs) from which the samples were taken, Individual batches processed every day were recorded (with an average of two batches used for sampling per day), so variable date was used as an approximation of farm of origin, to study the possible effect on microbial contamination. Samples were identified in four groups every day (from 1= at the beginning of the line to 4= at the end of the line) to identify the location of carcases on time during the day. 'Week' was used as an approximation of season (i.e. the week of the trial during which we collected the swabs (Week 1-5)) .

<u>Yersinia spp.</u>

In the final multivariable model there was a statistically significant association with the presence of *Yersinia* for the variable date, specifically the 29th of November 2011 ($p<0.001$). Two batches (i.e. two different farms) were processed on that day; one of the farms provided pigs for the rest of the trial and the other one only sent animals on that day. Otherwise, there was no statistical difference ($p=0.55$) in the *Yersinia* detection in carcases between the inspection methods.

Total aerobic plate count and *Enterobacteriaceae* count

Linear models were also applied to investigate the association between the inspection method and the microbial contamination of carcases.

The results indicate that the variable date influences the outcome (results not shown). This seems to imply that the batch/farm or origin is the only variable that influences the results in terms of aerobic plate count.

When we tested the category 'presence of *Enterobacteriaceae* (n=235 samples)', with the *Enterobacteriaceae* count as the outcome, the only statistical significant variable was the inspection method, implying that the inspection method is the only variable that influences the results in terms of *Enterobacteriaceae* count.

**Discussion**

The work described here used samples archived during an FSA study, FS145003 'Trial of visual inspection of fattening pigs from non-controlled housing conditions' which was designed to compare the contamination and cross-contamination of carcases subjected to traditional and visual inspection post slaughter. It did not have an invasive sampling protocol, neither faecal samples, lymph nodes, nor tonsils were collected. The carcases were swabbed on one side from the back to the head and these were the only samples tested.

We identified one *Y. enterocolitica* 4/O:3 in our study, a serobiotype that has been strongly associated with human disease (Bottone) and two *Y. enterocolitica* 3/O;5,27, also considered to be pathogenic for humans (Drummond, *et al*., EFSA, 2012). Although *Y. enterocolitica* 2/09 have been associated with human disease (Moriki *et al*., Fredriksson-Ahomaa *et al*.), we were unable to find evidence of *Y. enterocolitica* 2/O:? causing human disease. Three of our five *Y. enterocolitica* then, belong to serobiotypes known to cause human disease. We recovered six *Y. frederiksenii*, and findings in a study by Greenwood and Hooper (1987) suggested an association with *Y. frederiksenii* and gastro-intestinal disease in humans. The organism has been demonstrated in caecal contents of pigs at slaughter in Great Britain during 1999-2000 by McNally *et al*. (2004), where they accounted for 8% of all the *Yersinia* spp. isolated from pigs.

We used a commercial system to confirm the identity of our putative *Yersinia*. Previous work by Sharma *et al*., (1990) describes API20E having 97% and 78% success rate in identifying *Y. enterocolitica* and *Y. frederiksenii* respectively. The identification system used in GIRU uses many more biochemical tests and also serotypes the isolates, the latter being an indicator of pathogenic potential. The revision of our results was not, therefore, unexpected. It does highlight the questions that need to be asked and the decisions to be made at the beginning of future studies: To what level do bacteria need to be identified? What methods are to be used? At what point do we stop applying more detailed techniques? Will it be sufficient to identify *Yersinia* spp. using API20E or is a fuller identification needed? What are the results to be used for? What impact do the methods used have on the apparent prevalence estimates obtained? Can prevalence estimates really be compared between different studies?

We have not calculated overall prevalence estimates for our study because of the lack of independence between the individual swabs. This was evidenced in the results of the original survey where in the univariable analysis an association was seen between carcases

sampled on two dates that had more risk of presence of Yersinia than on the rest of the dates ($p<0.03$); an association that persisted in the multivariable analysis i.e. there was a statistically significant association with the presence of Yersinia for the variable date, specifically the 29th of November 2011 ($p<0.001$). Two batches (i.e. two different farms) were processed on that day; one of the farms provided pigs for the rest of the trial and the other one only sent animals on that day. The numbers of positive samples are too small to be able to examine for any potential clustering by serotype.

In the original study, we expected our carcass swabs to have a lower rate of recovery of *Salmonellae* and *Yersinia* spp. compared to that from faecal samples in published studies, but to be comparable to those from studies where similar carcass swab sampling techniques were used (Anon, 2011, Anon, 2012, Ortiz *et al*.).

Out of necessity, our study used a single abattoir for carcass sampling. Both anecdotally, from the veterinarians who carried out the fieldwork, and from the total aerobic plate and *Enterobacteriaceae* counts, it was clear this abattoir had a very high standard of hygiene. It was visually clean and there was a very good protocol of hygiene measures implemented by both the plant personnel and the FSA personnel. Both had a very good routine of washing and disinfecting hands and knives on the line. This was the norm and was not just due to the occurrence of the trial. This emphasis on good hygiene practice would result in a low level of cross contamination between carcases under normal circumstances. The microbiological outcomes of the primary study were not, therefore, totally unexpected. Despite this reassurance, the prevalence estimates from our culture results were low. Traditional microbiological techniques isolated 12 *Yersinia* and no *Salmonella*.

To validate these results, we have used PCR based methods to test stored samples for DNA specific for *Yersinia* and *Salmonella* rather than repeat the same culture based tests on the samples. The primary intention was to re-determine the presence/absence of *Yersinia* and *Salmonella* using a different method. Testing of our frozen sample aliquots did not identify any further samples containing *Yersinia* DNA or any *Salmonella* DNA. Our frozen samples, however, were not aliquots of sample enrichments and this may be an area for improvement in any future study planning.

It may, indeed, be better to plan to use ISO standard PCR based testing on enrichment samples first, then culture only those giving a positive signal in order to optimise resources. This approach would obviously need thorough validation. It was not, therefore, adopted in the original study (FS145003).

When interpreting the *Salmonella* results, it must be borne in mind that our treatment of the swabs differed slightly from the BS EN ISO 6579:2002 (British Standard Institution, 2002) and Health Protection Agency (2011b) method insofar as the carcass swab was not incubated with the pre-enrichment step. All other steps proceeded as the published methods. Bonardi *et al*., published the results of two abattoir studies in 2003 and 2013. In both, enrichment culture for Salmonella was carried out. The first study did not include the carcass swab in the pre-enrichment whilst the later study did and the yields of Salmonella were 9/150 (6.0%: Wald 95% confidence interval [C.I.] 3-11%) and 49/451 (10.9%: Wald 95% C.I. 8-14%) respectively. This indicates that the addition of the carcass swab to the pre-enrichment medium possibly increases detection rates, although it is not a statistically significant difference, with the caveat that there have to be *Salmonellae* present on the

carcass swab before enrichment. We did isolate *Yersinia* from the same samples, indicating that the stomaching process was sufficient to liberate bacteria from the swab into the diluent.

## SUMMARY OF OUTCOMES

From the studies that have been completed in this project we have achieved several outcomes:

- DNA based testing did not identify any further *Yersinia* nor any *Salmonella*.
- *Yersinia enterocolitica* and *Yersinia frederiksenii* recovered by culture from the original carcass swab samples were biotyped, serotyped and biochemically confirmed as *Yersinia*.
- We confirmed that the results from the original microbial investigations were valid. They reflect the microbiological status of the pigs sampled on the line and the good hygiene practices in place in this abattoir.

## RECOMMENDATIONS

From this additional molecular diagnostics study we can make the following recommendations:

1. For any study that involves identification of the bacterial isolates the questions that are to be asked within the study should be clearly identified first. i.e. Why are we doing this? What are we looking for? This will lead to the identification of the most appropriate diagnostic methods to use.
2. When identification of bacteria is required, then ISO standard PCR based testing for the specific bacteria could be used on enrichment samples first, followed by culture only of those samples that give a positive signal. This could optimise resource use. However, this approach would need thorough validation first via an appropriately designed trial.

# APPENDIX 1

## REFERENCES


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