

FS900284 Survey of the Microbiological Contamination of Cull Ewes and Prime Lamb at Slaughter in England and Wales

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Keywords: 1. Foodborne Disease and AMR, • Foodborne disease, • Products of animal origin, • Sampling

<https://doi.org/10.46756/001c.123776>

FSA Research and Evidence

The aim of this FSA-funded survey was to determine microbial contamination on sheep carcasses in abattoirs in England and Wales as the last survey of healthy sheep at slaughter in England and Wales was undertaken 20 years ago. This project was run in conjunction with surveys funded by Defra and the PATH-SAFE research programme aimed at determining prevalence of caecal carriage of pathogens and levels of antimicrobial resistance in sheep at slaughter.

Twelve out of 132 abattoirs in England and Wales were recruited, covering 28% of the annual national sheep slaughterhouse throughput. In total, 1282 carcass swab samples were collected over a 12-month period from February 2023 to January 2024. All samples were tested for the isolation and quantification of *Salmonella*, 366 were tested for *Campylobacter* and 103 were tested for *Enterobacteriaceae* and *E. coli*.

Salmonella was only detected in 7 (0.55%) of carcass swabs. Five isolates were confirmed as *S. enterica* subsp. *diarizonae* serotype 61:k:1:5, a sheep-adapted type with no confirmed association with human disease. The other two isolates were confirmed as *S. Typhimurium*. *Campylobacter* contamination was detected on 76 (20.8%) of carcasses tested. Prevalence of *E. coli* (88.4%) and *Enterobacteriaceae* (75%) was as expected in freshly slaughtered meat, with 81.6% and 84.5% of these samples, respectively, presenting low levels of contamination.

The contamination detected on sheep carcasses was significantly lower than that found in caecal samples examined in the Defra study suggesting that slaughtering processes are effective in reducing carcasses being contaminated with pathogens. Correct cooking and hygienic handling and preparation of sheep meat will reduce/eliminate surface microbiological contamination further and reduce the risk to consumers. These results provide valuable baseline data that will help inform risk assessments and risk modelling and will inform future decisions by Government and the meat industry.

Acknowledgements

This survey of the microbiological contamination of cull ewes and prime lamb at slaughter in England and Wales was carried out by the Animal and Plant Health Agency. The work was undertaken by members of the Bacteriology Department and the Department for Epidemiological Sciences.

The authors would like to thank all the staff at the participating abattoirs without whom this work would not have been possible. We would also like to thank all the FSA field staff for their efforts in collecting the samples.

1. Lay Summary

Both government and industry recognise the importance of ensuring that fresh meat is safe for consumers by monitoring and taking steps to avoid contamination with organisms capable of causing foodborne illness during the slaughter process. Although sampling of carcasses and meats by food business operators occurs regularly, the more detailed sampling performed during this project enables better understanding of the baseline levels of bacteria in meat (for future monitoring purposes), helps to determine changing patterns of potential pathogens in the food chain over several years and helps identify emerging threats to public health.

The aim of this study was to establish the microbiological contamination found on culled ewe (sheep over 12-months old) and lamb (sheep under 12-months old) carcasses in abattoirs in England and Wales. The last survey of microorganisms on sheep carcasses in England and Wales was carried out in 2003. The results from this latest survey will provide the FSA with an understanding of the baseline microbiological contamination of sheep carcasses. The data generated can inform future microbiological risk assessments that may help with future trade negotiations.

In the study, we investigated the levels of contamination by two of the leading causes of foodborne bacterial disease in the UK, namely *Salmonella* and *Campylobacter*. We also determined levels of non-disease-causing bacteria, *E. coli* and *Enterobacteriaceae*, that are typically carried in the intestines of most animals and so their numbers on carcase surfaces are used as indicators of hygiene in the food industry. The work was carried out in conjunction with two other government funded projects that looked at *Salmonella* and *Campylobacter* carriage in the intestines of sheep. Over half of the carcase swabs had “matched” caecal samples i.e., taken from the same sheep. The combined results give a detailed picture of the bacteriological status of sheep at slaughter in England and Wales in 2023/2024.

A total of 1282 carcase swabs (1078 lamb, 180 ewe, 24 unspecified) were taken from 12 abattoirs in England and Wales during the period February 2023 to January 2024. All were tested for *Salmonella*, 366 (297 lamb, 65 ewe, 4 unspecified) were tested for *Campylobacter* and 103 (81 lamb, 17 ewe, 5 unspecified) were tested for *Enterobacteriaceae* and *E. coli*. The results show very few carcasses were contaminated with *Salmonella*. Only 4 lamb samples (0.4%) and 3 ewe samples (1.6%) were positive. Only two samples (both from lambs) contained types of *Salmonella* known to cause disease in people. *Campylobacter* was more widespread and was detected in 76 of the 366 (20.8%) swabs tested. The positives swabs came from 65/297 lamb carcasses (22%) and 10/65 ewe carcasses (15%) (plus one from an 'age unspecified' carcass). The types found have the potential to cause disease in people although they are not well able to survive in air, so the numbers are likely to drop from abattoir to retail to homes. The cooking process should eliminate most of the bacteria present.

Some level of contamination with intestinal bacteria is almost inevitable following the slaughter and evisceration processes but the aim of hygienic slaughter is to prevent spillage of intestinal contents and minimise carcase contamination. Prevalence of *E. coli* and *Enterobacteriaceae* was high, but the levels were mostly low. For *E. coli* 91/103 (88.4%) samples had detectable levels (89% and 88.2% of lamb and carcase samples respectively) and 77/103 samples (75%) had detectable *Enterobacteriaceae* (74% and 76% of lamb and ewe carcase samples respectively). There was no significant difference in rates and levels of contamination between lamb and ewe carcase samples. From all swabs tested, 40% and 32% had low levels (fewer than 100 bacterial counts per swab) of *Enterobacteriaceae* and *E. coli* respectively, 44.6% and 49.5% had medium levels (101-9999 counts per swab), 15.5% and 18.4% had high levels (10000 or greater). A high prevalence of hygiene indicator bacteria is expected in freshly slaughtered meat.

This study has produced a valuable data set that gives a good indication of the microbial quality of sheep at slaughter in England and Wales. There were no significant differences in contamination rates between lambs and ewes. Encouragingly, even though there was a relatively high prevalence of *Salmonella* (8.39%) in the caeca samples of sheep (determined in the associated Defra funded project), the prevalence of *Salmonella* on sheep carcasses was very low (0.5%). Similarly, the prevalence of *Campylobacter* on sheep carcasses (20.8%) was also lower in comparison to sheep caecal samples (46.8%). This suggests that the slaughtering, hide removal and evisceration processes are effective in preventing extensive carcase contamination with pathogenic *Salmonella* and *Campylobacter*. The low levels of indicator bacteria found on carcasses sampled suggests that good hygienic practices are being carried out during lamb and sheep slaughtering. High numbers of *Campylobacter* and indicator organisms

on some carcasses suggest there is potential for improved practices at slaughter and further study may be warranted. However, correct storage and cooking would be expected to reduce or eliminate any surface microbiological contamination that maybe present.

These results provide valuable baseline data that will help inform risk assessments, risk modelling and will help to inform future decisions by Government and the meat industry.

2. Executive Summary

Both government and industry recognise the importance of ensuring that fresh meat is safe for consumers by monitoring and taking steps to avoid contamination with organisms capable of causing foodborne illness during the slaughter process. Although sampling of carcasses and meats by food business operators occurs regularly, the more detailed sampling performed during this project enables better understanding of the baseline levels of bacteria in meat (for future monitoring purposes), helps to determine changing patterns of potential pathogens in the food chain over several years and helps identify emerging threats to public health

The last microbiological survey of healthy sheep presented for slaughter in England and Wales was undertaken 20 years ago and there have been no *Salmonella* prevalence studies of sheep undertaken in the UK since then, nor any assessment of *Campylobacter* prevalence. Up-to-date data are required to help inform future industry and government decision making. This project was run in conjunction with surveys funded by Defra and by the [PATH-SAFE](#) research programme which were aimed at determining prevalence of caecal carriage of *Salmonella* and *Campylobacter* as well as levels of antimicrobial resistance (AMR) in slaughter sheep. The aim of this FSA-funded survey was to establish microbial contamination on culled ewe (sheep over 12-months-old) and lamb (sheep under 12-months-old) carcasses in abattoirs in England and Wales. Specifically, we tested for the presence and levels the two most common causes of foodborne bacterial disease (*Salmonella* and *Campylobacter*), and commensal *E. coli* and *Enterobacteriaceae* that are typically used as indicators of hygiene in the food industry.

Twelve out of a possible 132 abattoirs in England and Wales were recruited for the study in spring 2023. The cohort abattoirs covered a range of geographical locations, processing practices and throughputs. They covered approximately 28% of the annual national sheep slaughterhouse throughput for England and Wales. A total of 1282 swab samples (1078 lamb, 180 ewe, 24 unspecified) were collected by FSA staff during the period February 2023 to January 2024. All samples were tested following ISO standard methods for the isolation and quantification of for *Salmonella*, 366 (297 lamb, 65 ewe, 4 unspecified) were tested for

Campylobacter and 103 (81 lamb, 17 ewe, 5 unspecified) were tested for *Enterobacteriaceae* and *E. coli*. Species confirmation was achieved by Whole Genome Sequencing for *Salmonella* and by Maldi-ToF for *Campylobacter*.

The results showed that *Salmonella* was only detected in seven out of 1282 carcase swabs tested (0.5%), including four out of 1078 (0.4%) from lambs and three out of 180 (1.7%) from ewes (no significant difference observed). Five of the isolates detected were confirmed as *S. enterica* subsp. *diarizonae* serotype 61:k:1:5 which is a sheep-adapted type with no confirmed association with human disease. Two lamb isolates were confirmed as *S. Typhimurium* which is the second most common type associated with human disease in the UK.

Campylobacter contamination was detected in 76 out of 366 carcase swabs tested (20.8%). There was no significant difference in rates of *Campylobacter* contamination between lambs and ewes, with 65 of 297 lamb carcasses (22%) and 10 of 65 ewe carcasses (15%) testing positive (plus one from an 'age unspecified' carcase). The predominant *Campylobacter* species detected was *C. jejuni* (n=71; 96%) with the rest being *C. coli* (n=3) and *C. fetus* (n=1). One carcase was positive for both *C. jejuni* and *C. coli*. *C. jejuni* and *C. coli* are the most common types to cause disease in people although they are not well able to survive in air, so the numbers are likely to drop from abattoir to retail to homes. Correct cooking processes should eliminate most of the bacteria present including *Salmonella* and *Campylobacter*.

Some level of contamination with intestinal bacteria is almost inevitable following the slaughter and evisceration processes but the aim of hygienic slaughter is to prevent spillage of intestinal contents and minimise potential carcase contamination. Prevalence of *E. coli* and *Enterobacteriaceae* was high. For *E. coli* 91 of 103 (88.4%) samples had detectable levels (89% and 88.2% of lamb and ewe carcase samples respectively) and 77 of 103 samples (75%) had detectable *Enterobacteriaceae* (74% and 76% of lamb and carcase samples respectively). There was no significant difference in prevalence and levels of contamination between lamb and ewe carcase samples. From all swabs tested 40% and 32% of samples had low levels ($<10^2$ cfu/swab) of *Enterobacteriaceae* and *E. coli* respectively, 44.6% and 49.5% had medium levels (10^2 - 10^4 cfu/swab), 15.5% and 18.4% had high levels ($>10^4$ cfu/swab). A high prevalence of hygiene indicator bacteria is expected in freshly slaughtered meat.

In conclusion, this study has produced a valuable dataset on the microbiological contamination found on the carcasses of sheep at slaughter in England and Wales in 2023/2024. There were no significant differences in contamination between lambs and ewes. Encouragingly, even though

there was a relatively high prevalence (8.4%) of caecal carriage (determined in the associated Defra funded project), levels of carcase contamination with *Salmonella* are very low (0.5%). Furthermore, despite the prevalence of *Campylobacter* carcase contamination being higher than *Salmonella* (20.8%) there was still a notably lower prevalence compared to that in caeca (46.8%). This suggests that the slaughtering processes are effective in preventing extensive carcase contamination with pathogenic *Salmonella* and *Campylobacter*. The low levels of indicator bacteria found on most carcasses sampled suggests that good hygienic practices are being carried out during the slaughtering of lamb and sheep. High numbers of *Campylobacter* and indicator organisms on some carcasses suggest there may be potential for improved practices at slaughter. However, correct storage and cooking would be expected to reduce or eliminate any surface microbiological contamination that maybe present.

These results provide valuable baseline data that will help inform risk assessments, risk modelling and will help to inform future decisions by Government and the meat industry.

3. Introduction

The microbiological quality of fresh meat is a public health concern due to the potential for contamination with organisms able to cause foodborne illness in consumers. During the slaughtering phases carcasses of livestock intended for human consumption can be contaminated with both pathogenic and commensal microorganisms. One of the main objectives within the [FSA's Strategy for 2022-2027](#) is ensuring that 'food is safe'. A key component for this is monitoring pathogenic microbiological hazards through surveillance. Surveys provide a 'snapshot' of the level and type of microbiological contamination in foods at various points in the food chain. This is important for assessing the potential consumer exposure to these hazards (a key component of microbiological risk assessments) from the consumption of raw and/or undercooked foods but also through cross-contamination events when these foods are stored and handled unhygienically. Surveillance allows new baselines for foodborne bacteria in foods to be set (for future monitoring purposes), and enhances understanding of the changing patterns of potential pathogens in the food chain over several years and the identification of emerging threats to public health. The abattoir is one of the primary steps in the 'farm-to-fork' process and surveillance of pathogens will allow more effective implementation of control strategies by risk managers (Milnes et al., 2008).

Campylobacter and non-typhoidal *Salmonella* are the two most common bacterial agents of foodborne illness in the UK. Between 1992 and 1999 it was estimated that 16% of cases of human infectious intestinal disease were related to consumption of red meat with *Campylobacter*, *Salmonella*

and Shiga toxin-producing *Escherichia coli* (STEC). Moreover, in the UK, there have been 517 outbreak associated human cases of *Salmonella* which have been attributed to consumption of lamb and beef products between 2015 and 2020, most due to *Salmonella* Typhimurium (DEFRA, 2021). Almost 300 of these cases were from a single outbreak relating to ovine meat and products ([Almost 300 sick and one dead due to Salmonella in UK | Food Safety News](#)). The last microbiological survey of healthy sheep presented for slaughter in England and Wales was undertaken by APHA in 2003 (Milnes et al., 2008). This study investigated *Salmonella* faecal carriage in sheep. There have been no *Salmonella* prevalence studies of sheep undertaken in the UK since then, nor any assessment of *Campylobacter* prevalence.

Variations in the *Salmonella* serovars isolated, animal husbandry, slaughter and carcase processing, marketing and the public's consumption of lamb and mutton, have been observed since the 2003 survey was undertaken. Notably, in the UK between 2017 and 2018, there were almost 300 outbreak-associated human cases of a new *Salmonella* Typhimurium strain attributed to consumption of ovine meat or products. The strain was identified through the Animal and Plant Health Agency's (APHA) scanning surveillance activities following postmortem examination of sheep in a flock where a high level of mortality had occurred. Phylogenetic comparison of these sheep isolates with human isolates identified an epidemiological link (Carson & Davies, 2018). The outbreak generated media attention due to the numbers of cases, including a death, in which *Salmonella* infection was considered a contributing factor.

It is vitally important to have up-to-date information to inform risk assessments, control measures and advice to protect public health. Although sampling of carcasses and meats occurs regularly through the implementation of the meat hygiene legislation (Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs, adopted by UK legislation), the testing is carried out by the Food Business Operator's (FBO's) and the FSA do not have sight of the results. Up-to-date data are required regarding the level of contamination on sheep carcasses for *Campylobacter*, as the contribution of ruminants to human campylobacteriosis is not well assessed. Furthermore, quality maintenance is important to ensure consumer confidence and health protection. Up-to-date data regarding the general microbiological quality of slaughtered animals will lead to improved risk assessments and risk management advice and help to ensure consumer safety and confidence.

The survey detailed here was run in conjunction with surveys funded by Defra and by the [PATH-SAFE](#) research programme which were aimed at determining prevalence of caecal carriage of *Salmonella* and

Campylobacter as well as levels of antimicrobial resistance (AMR) in slaughter sheep in premises recruited from a list of FSA-registered abattoirs in England and Wales.

The specific aims of the survey were:

1. To determine the prevalence of *Salmonella* spp. contamination on cull ewe and lamb carcasses
2. To gather quantitative data on the levels of *Salmonella* present in positive caecal samples (from the Defra *Salmonella* survey) and carcass swabs
3. To establish the presence and levels of *Campylobacter* on cull ewe and lamb carcasses
4. To gather data on the levels of *E. coli* and *Enterobacteriaceae* found on cull ewes and lamb carcasses.

4. Materials and Methods

4.1. Recruitment of abattoirs

In December 2022, we approached 132 FSA-registered abattoirs within England and Wales which slaughter ovines to volunteer to participate in this survey. The recruitment letter explained the rationale for the study and requested participation on an opt-in basis. Upon recruitment a follow-up telephone or Microsoft Teams call was organised either with the abattoir technical lead or owner to explain further details. At the end of the call, it was confirmed verbally that the abattoir consented to participate in the survey. This was followed by written confirmation. FSA Official Veterinarians were identified to act as points of contact. Abattoirs received a standard operating procedure (SOP) document with details on how to collect samples, package the boxes and fill in the sampling form (Appendix 1). Abattoirs were then given their sampling schedule for the study. Demographic data including throughput of ovines over and under 12-months-of-age which was not held by APHA or FSA was requested from the abattoirs to produce appropriate sampling schedules.

Six abattoirs were recruited in January 2023 and a pilot study in one of them was run in the following month. Three premises began sampling in February 2023 and three began in March 2023. One abattoir collected samples only for 3 months before leaving the study in May. A further six abattoirs were recruited, joining the study in May 2023 (n=5) and July 2023 (n=1).

4.2. Sampling

Sampling kits in BioTherm transport boxes (Intelsius, York, UK) were assembled at APHA, Weybridge, and dispatched to abattoirs prior to sample collection. Each box contained Biochill freezer packs (frozen at the abattoirs at least 48 hrs before sampling), gloves, labels, swabs and sample pots and included a reminder of a breakdown of the samples to be taken from sheep who were over 12 months and those who were under 12-months and key sampling procedures. A sample collection form was also included (Appendix A). Sample collection was undertaken by FSA staff. Sterile, Polywipe™ swabs pre-moistened in saline buffer were used to swab carcasses (product MW726, MWE, UK) following FSA standard protocols (Appendix B). One animal per batch was sampled with gloves changes between each sample taken. Where matched caecal and carcass samples were taken FSA staff tagged carcasses using cable ties and laminated labels to enable identification as samples were taken at different points on the slaughter line. Samples were refrigerated after collection and packaged later the same day with freezer packs and returned to APHA. Staff were also asked to capture details relating to age, gender, kill no, weight, line speed, origin, method of slaughter by filling in sampling forms. In most instances, one sheep per batch arriving at the abattoir was randomly selected for sampling but for large batches one in 25 carcasses were sampled. Samples were delivered by courier to APHA at controlled temperatures (2-8°C) the day after collection.

4.3. Schedule

Sampling was conducted over a 12-month period between February 2023 and January 2024. For the purposes of this study abattoirs were given the following definition:

Over 12-months-old (cull ewes): adult animals which have a permanent incisor erupted through the gums.

Under 12-months-old (lambs): young animals lacking permanent teeth.

Abattoir throughput data for 2022, broken down by under and over 12-months-old sheep, was used to determine the random sampling schedule. The number of animals to be sampled from within the population and the abattoirs to which they belonged were randomly allocated by month and abattoir using the “tidyr” library in R. Slaughter throughput per month for all abattoirs was examined using historical data and found to be relatively stable year on year. This assumption was further explored and deemed appropriate by abattoir managers and checked retrospectively once data became available early in 2024.

The sampling schedule was run for the first time in January 2023 and then was re-run in late April and late June of the same year to accommodate new abattoirs enrolling in the study. At each re-run the number of samples scheduled to be collected was set as the total number to be collected over the whole study minus the samples already collected.

Sampling days were randomly assigned to the abattoir based on days they regularly processed ovines, omitting bank holidays and Fridays. A maximum of 15 samples arriving at the laboratory on any day was set to ensure consistency between sampling occasions. Second and occasionally third dates were assigned to any sampling occasions with numbers exceeding this figure. [Figure 1](#) shows the planned sampling over the study and the throughput of cohort abattoirs.

Abattoirs were given the schedule at the start of the study with monthly reminders. If any sampling occasions were missed a new date acceptable to laboratory and abattoir was assigned. If they couldn't make that date a third attempt was made. Occasionally, and particularly at the beginning of the study this meant rolling over to the next month.

The allocations to each abattoir were proportional to their throughput of ovines aged over and under 12-months-old. There were a few abattoirs who noted occasionally slaughtering animals over 12-months-old but in too few numbers to be included in the schedule. There were also occasions where some abattoirs had too few batches of lambs and made up their "sampling quota" with an animal over 12 months.

A sample size of 1200 sheep was calculated based on an estimated design prevalence of *Salmonella* of 3% to give an accuracy of approximately 1% with 95% confidence. Actual Agresti-Coull confidence intervals for this design prevalence would be 2.2-4.1. An extra 15% of samples were included to allow for issues around the sampling, courier and testing etc., giving a final sample size of 1380.

4.4. Sample processing

4.4.1. Sample receipt and swab processing

Upon receipt at APHA Weybridge boxes and contents were checked for arrival time, sample damage and internal temperature. To satisfy confidentiality concerns no data pertaining to abattoir or animal ID were stored. Any information pertaining to animal ID was checked on arrival (anomalies identified, such as multiple samples from same animal or multiple animals per batch) and then recoded with new, unique APHA sample identification codes for anonymity. Samples were processed by the laboratory within 24 hrs of receipt. Swabs were removed from packaging using sterile forceps and placed inside a stomacher bag along with 40ml sterile Buffered Peptone Water (BPW). (For an initial period, swabs were

added to 30ml BPW based on calculations of minimal amount needed to give maximum sensitivity in all the testing undertaken. However, it was not until the first two *Salmonella* positive swabs were identified (from samples taken in May 2023) that it was realised 30mls was insufficient for *Salmonella* enumeration due to fluid retention and losses when transferring and splitting between different processing stages. The volume was subsequently increased to 40mls.) The swabs were processed in a Stomacher® 400 Circulator Lab Blender (Seward, UK) for 30 seconds. The resulting final suspension was split into two sterile containers: 12mls for microaerobic testing (*Campylobacter* culture) and 28mls for aerobic testing (*Salmonella*, *E. coli*, and *Enterobacteriaceae* culture).

4.4.2. *Salmonella* isolation and enumeration

Salmonella was isolated following ISO 6579:1-2017. Briefly, 10mls of swab sample were added to 90mls BPW (1:9, v:v) and enriched at $37 \pm 1^\circ\text{C}$ for 18 ± 2 hrs. The remainder of the suspension was then used for *E. coli* and *Enterobacteriaceae* quantification on select samples and the remainder stored at 4°C . From the enriched BPW 0.1 ml was inoculated onto modified semi-solid Rappaport-Vassiliadis agar (MSRV; Mast: Mast Group Ltd, Bootle, UK) containing 1mg/ml novobiocin (Sigma-Aldrich, Dorset, UK) and incubated at $41.5 \pm 1^\circ\text{C}$ for 24 ± 3 hrs. Using a 1 μl loop, a subculture was taken from the edge of spreading growth on MSRV, inoculated onto Rambach agar (Merck Millipore, Watford, UK) and Brilliant Green Agar (Sigma-Aldrich, Dorset, UK) + novobiocin and incubated at $37 \pm 1^\circ\text{C}$ for 24 ± 3 hrs. MSRV plates were returned to the incubator for a further 24 ± 3 hrs. Any MSRV plates where growth had substantially spread between 24 and 48 hrs of incubation were sub-cultured again onto Rambach and BGA agar. BGA was used in addition to Rambach as *S. enterica* subsp. *diarizonae*, a sheep-associated type, often has an atypical (for *Salmonella*) appearance on Rambach. Preliminary confirmation as *Salmonella* was achieved by slide agglutination using poly-O, poly-H and O61 antisera. The latter was used to aid identification of *S. enterica* subsp. *diarizonae*, which poorly agglutinated with poly-O antiserum. Where a sample was positive, the non-enriched swab suspension was retrieved from cold storage for quantitative bacteriology using the MPN method (ISO6579-2:2012). The protocol follows the basic methodology used for isolation (outlined above). For each sample, 2.5mls of the 'neat' dilution (i.e. the starting suspension made above) was added to three wells of a 12 well tissue culture plate. Three x 1/5 serial dilutions were then made by removing 0.5ml of suspension and adding to 2mls BPW in the next column of wells etc to give a test plate of three replicates at four dilutions. Plates were incubated as above and then 20 μl removed from each well and transferred onto the surface of MRSV agar in 12-well plates. Subsequent steps follow the isolation protocol detailed above. A score was produced by counting the number of positives at each dilution and then an MPN value (colony forming units - cfu) was

determined using an online calculator ([ISO Standards Maintenance Portal](#)). Levels of *Salmonella* in caecal samples were also determined following this method except faeces were removed from cold storage and a starting 1/10 suspension made by homogenising 1 g in 9ml BPW. The limits of quantification were 6 cfu/swab for carcase samples and 1.6 cfu/g for caecal contents.

Salmonella confirmation and serotyping was achieved by Whole Genome Sequencing (WGS). Briefly, genomic DNA was extracted with a KingFisher MagMAX™ CORE instrument and the MagMAX™ CORE Nucleic Acid Purification Kit (Thermo Fisher Scientific, UK) from overnight LB broth cultures of single colonies. Libraries were normalised and pooled before running on an Illumina NextSeq 500/550 instrument to generate 150 base pair paired-end reads. Sequenced isolates were analysed with an APHA in-house in silico serotyping pipeline to confirm the serovar (APHA, 2023. NextflowSerotypingPipeline. APHA-BAC. Available at <https://github.com/APHA-BAC/NextflowSerotypingPipeline>.

4.4.3. *Campylobacter* isolation and enumeration

To detect and enumerate *Campylobacter* spp., a procedure based on ISO10272-1:2017 was used. Swab suspensions were inoculated onto modified charcoal cefoperazone deoxycholate agar (mCCDA). One ml volume of suspension was plated across three mCCDA plates. Additionally, 100 µl of homogenate were plated onto single plates of mCCDA and Butler's agar. All plates were incubated in a microaerobic atmosphere at 41.5± 1°C for at least 44 hours.

Putative *Campylobacter* colonies were counted on the mCCDA agar plates and were picked and sub-cultured onto 7% sheep blood agar and incubated in a microaerobic atmosphere at 41.5± 1°C. The detection limit for quantification was 30 or 40 cfu sample (depending on the initial volume added to the swabs - see 6.3.1). To detect lower levels, 10mls of sample suspension were added to 90mls of Bolton broth and incubated in an microaerobic atmosphere at 41.5°C for 44 hours ± 4 hours. Enriched culture (10µl) was plated onto mCCDA plate and Preston/Butzler agar and incubated for 48 hours. Up to five suspect colonies were selected and sub-cultured onto 7% sheep blood agar and incubated in a microaerobic atmosphere at 41.5± 1°C. *Campylobacter* genus and spp. identification was confirmed by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight) Mass Spectrometry (MALDI-ToF) of the pure cultures using Bruker Biotyper Sirius or Microflex LT/SH MALDI-ToF machines and collected spectra identified with Bruker MBT Compass 4.1.100.

4.4.4. *E. coli* enumeration

To detect and enumerate *E. coli*, ISO 7251:2005 was followed (Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of presumptive *E. coli* — most probable number technique). For each swab sample a 1:4 serial dilution series was made in triplicate by transferring 2 ml of sample, using a sterile pipette, to test tubes containing 8 ml of Lauryl Sulphate Broth (LSB, Neogen). This was repeated until a 10^{-4} dilution of the initial sample suspension was achieved. LSB test tubes were incubated at $37 \pm 1^\circ\text{C}$ for 24 ± 3 hrs. Test tubes were returned to the incubator for a further 24 ± 3 hrs. Using a 10 μl loop, any exhibiting opacity, cloudiness or gas formation in LSB between 24 and 48 h of incubation were used to inoculate 10 ml test tubes of EC Broth (ECB, Thermo Scientific, Hemel Hempstead, UK). ECB test tubes were incubated at $44 \pm 1^\circ\text{C}$ for 24 ± 3 hrs and returned to the incubator for a further 24 ± 3 hrs. Using a 10 μl loop, tubes exhibiting opacity, cloudiness or gas formation in ECB between 24 and 48 h of incubation were used to inoculate corresponding 5 ml of Peptone Water NutriSelect Plus (Merck: Merck Millipore, Watford, UK). Bijous containing peptone water were incubated at $44 \pm 1^\circ\text{C}$ for 48 hrs $\pm$ 6 hrs. After incubation, 1 μl from each peptone water was spotted, using a sterile loop, onto filter paper pre-moistened with James' Reagent (BioMerieux) to check for indole production. For any replicates containing peptone water that did not exhibit indole-production via spotting, 500 μl of Kovac's Reagent (Merck) was added to further indicate any presence of indole. Presence and/or absence of indole production and gas formation in ECB was recorded and used to generate a MPN of presumptive *E. coli* ([ISO Standards Maintenance Portal](#)). The detection limits were 2.7×10^1 – 1.4×10^4 cfu/swab.

4.4.5. Enterobacteriaceae enumeration

To detect and enumerate *Enterobacteriaceae*, ISO 21528-2:2017 (Microbiology of the food chain - Horizontal method for the detection and enumeration of *Enterobacteriaceae* Part 2: Colony-count technique) was followed. Briefly, 1 ml of swab sample was pipetted into a sterile petri dish and overlaid with 15 ml of molten Violet Red Bile Glucose Agar (VRBG) (Oxoid, ThermoFisher, UK). Two extra plates were also prepared using 0.1ml swab sample and 0.1ml of swab sample diluted 1/10. Once set, a 5 ml cap of VRBG was added and plates were incubated at $37 \pm 1^\circ\text{C}$ for 24 ± 3 hrs. Using a 1 μl loop, 1 to 6 presumptive *Enterobacteriaceae* colonies were sub-cultured onto Nutrient agar plates and incubated at $37 \pm 1^\circ\text{C}$ for 24 ± 3 hrs. After incubation, presumptive colonies were streaked onto filter paper pre-moistened with oxidase reagent (Biomérieux) and any oxidase negative colonies were then inoculated into 10 ml Glucose OF medium (Neogen) which was overlaid with 2 ml of mineral oil (ThermoFisher, UK) and incubated for $37 \pm 1^\circ\text{C}$ for 24 ± 3 hrs. Colonies which were oxidase

negative and glucose-positive were presumed to be *Enterobacteriaceae* and any characteristic colonies were counted. The detection limits were 4×10^1 – 1.3×10^6 cfu/swab.

5. Results

5.1. Samples

Twelve abattoirs out of a possible 132 (9.1%) in England and Wales were recruited for at least part of the survey with a combined annual ovine throughput in 2023 of 3,519,954 sheep (28.2%) compared to 12,492,243 for all abattoirs in England and Wales as a whole. The cohort abattoirs ranged in throughput from 3,000 to 756,000 sheep slaughtered annually (mean of 293,330 and median of 140,654). Actual throughput numbers have been rounded to the nearest 500 to allow anonymity. Two abattoirs reported slaughtering only under-12-month lambs and another three abattoirs reported slaughtering approximately 33% of their total throughput as over-12-month sheep. Three abattoirs slaughtered only sheep on any given day, three slaughtered cattle and sheep on the same days, and six slaughtered sheep, cattle and pigs.

A total of 1380 carcase samples were originally planned to be collected across the 12 abattoirs, which consisted of a target of 1200 plus 180 (15%) as contingency. In the end, 1282 (1078 lamb, 180 ewe, 24 unspecified) were collected and eligible for testing. [Table 1](#) gives the scheduled number of samples to be collected compared with the numbers collected from each abattoir. In the first month of study (February 2023) and June 2023, several boxes of samples arrived at the laboratory above the required temperature (2-8 °C). In February 2023 this was largely down to incorrect packaging, so abattoirs were given extra information on packaging samples. In June 2023 there was a heatwave so extra freezer packs were subsequently added to compensate. All carcasses sampled were fit for human consumption with no signs of clinical disease. The majority of carcasses sampled (93.7%; 1201/1282) were halal slaughtered.

Of the 1282 carcase samples, 772 had a matching caecal sample taken from the same animal (collected for the Defra *Salmonella* survey).

Recruited abattoirs varied in geographical location. To identify geographical biases in sheep presented for slaughter at the cohort abattoirs the location of all premises sending sheep to slaughter in 2023 to cohort abattoirs were mapped ([Figure 1](#)). Data relating to two abattoirs could not be found so [Figure 1](#) is therefore an underestimate of the study population. Despite limited numbers of abattoirs taking part, all areas of the country populated with sheep were covered in the survey and the maps show good visual correlation. Although only abattoirs in England and Wales took part, some of these premises did receive sheep from Scotland.

Table 1. Monthly sampling at abattoirs as a proportion of monthly throughput (number of ovines) in England and Wales in 2023/2024

Sampling period	No. abattoirs sampling	Actual vs. planned carcase swab samples	Throughput of cohort abattoirs	Monthly contribution to throughput for cohort (%)	Throughput in all abattoirs**	Monthly contribution to throughput for all (%)
Feb 2023	6	12/29	206,646	6.93	934,297	7.48
Mar 2023	6	67/64	251,080	8.42	1,215,143	9.73
Apr 2023	6	56/53	177,780	5.96	965,012	7.72
May 2023	10*	57/75	194,182	6.51	946,140	7.57
Jun 2023	10*	81/91	224,253	7.52	1,099,690	8.80
Jul 2023	11	123/145	259,559	8.70	932,829	7.47
Aug 2023	11	117/156	305,522	10.24	1,039,153	8.32
Sept 2023	11	163/156	305,297	10.23	1,009,534	8.08
Oct 2023	11	175/179	324,469	10.88	1,110,423	8.89
Nov 2023	11	159/139	251,608	8.44	1,126,971	9.02
Dec 2023	11	162/151	262,997	8.82	1,129,764	9.04
Jan 2024	11	110/110	219,499	7.36	983,287	7.87
Total		1282/ 1348	2,982,892	100	12,492,243	100

*One abattoir left the study in May 2023, and another recruited in June 2023

**132 FSA-registered abattoirs in England and Wales

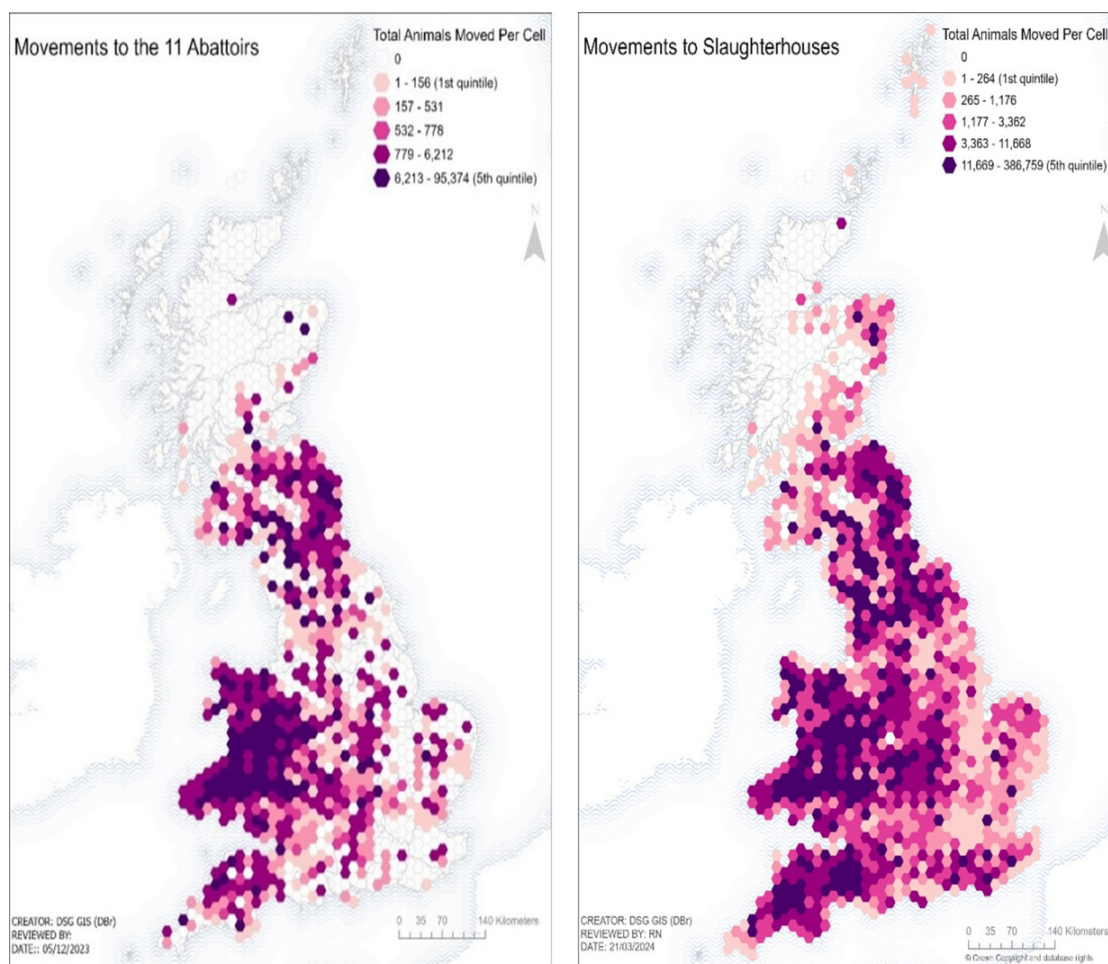


Figure 1. Maps showing the source location for, on the left, a) sheep entering the cohort abattoirs and on the right b) sheep entering all abattoirs in England and Wales in 2023. Data source: DEFRA Animal Movement Licensing System (AMLS) and Livestock Demographic Data Groups (LDDG).

5.2. *Salmonella* isolation and enumeration

A total of 1282 carcase swabs (1078 lamb, 180 ewe, 24 unspecified) were tested for the presence of *Salmonella*. Of these, only seven had detectable *Salmonella*, giving an overall annual prevalence of 0.55%. There was a difference in prevalence between lamb and ewe samples with four positive swabs from lamb carcasses (0.4%) and 3 from ewes (1.6%) but this was not significant ($p=0.0668$, Fisher's exact test). The prevalence of 0.55% was considerably lower than that found for caecal samples tested as part of the associated Defra funded project OZ0348 where 103/1228 caecal samples were found to be positive (8.39%). Details of the carcase isolates are shown in [Table 2](#). The seven isolates were: *Salmonella enterica* subsp. *diarizonae* serovar 61:k:1,5,7 ($n=5$), *S. Typhimurium* ST19 ($n=1$), and a monophasic *S. Typhimurium*, 4,12:i:- ($n=1$). The positive carcase swabs were from five different abattoirs one of which had three positives (in samples taken in May, October and December 2023).

Table 2. Details of the *Salmonella* isolates and the corresponding carcase and abattoir throughput information.

Sample ID	Sampling period	Salmonella Serovar	MPN	Ovine Age (months)	Carcase weight (kg)	Abattoir throughput (ovines/year)
S341	May 2023	61:k:1,5,7	ND	>12	22	>500,000
S397	May 2023	61:k:1,5,7	ND	>12	26	>500,000
S3536	Oct 2023	61:k:1,5,7	<6	>12	49.7	>500,000
S3624	Nov 2023	61:k:1,5,7	1100	<12	18	>500,000
S3674	Nov 2023	<i>S. Typhimurium</i> ST19	6.1	<12	18	>500,000
S3932	Dec 2023	61:k:1,5,7	720	<12	22	>500,000
S4009	Jan 2024	Monophasic <i>S. Typhimurium</i> 4,12:i:-	<6	<12	25	100,000-500,000

MPN (Most Probable Number) – estimate of the levels of *Salmonella* contamination (cfu/swab). ND – not determined due to insufficient sample.

The levels of *Salmonella* on carcase swabs were quantified by an MPN method. Two were negative by this method and only positive after enrichment indicating low levels (<6 cfu/swab) and one was just over the lower detection limit (6.1 cfu/swab). Higher levels of contamination were seen in the other two positives (720 and 11 cfu/swab), both of which were *S. enterica* subsp. *diarizonae* serovar 61:k:1,5,7.

The levels of *Salmonella* were also quantified in 60 positive caecal samples (27 lamb, 31 ewe, 2 unspecified). Levels ranged from < 1.6 cfu/g to > 4.4 x 10⁴ cfu/g (Table 3). Nine of 27 lamb samples (33%) and 12/31 ewe samples (39%) had levels below the detection limit (1.6 cfu/g). Twenty-one samples had levels below the MPN detection limit of <1.6 cfu/g: 9/27 from lambs (33%), 12/31 from ewes (39%). Seven samples had levels above the upper quantifiable limit of 4.4 x 10⁴ cfu/g: 2/27 from lambs and 5/31 from ewes. There was no significant difference in the levels between the two age groups (p=0.2739, t test).

Table 3. Levels of *Salmonella* in lamb and ewe caecal samples (cfu/g caecal contents).

Caecal levels of <i>Salmonella</i> (cfu/g)	Numbers of lamb samples (%)	Numbers of ewe samples (%)
<1.6	9 (33)	12 (38.7)
1.6-100	9 (33)	6 (19.4)
10 ² -10 ³	5 (18.5)	6 (19.4)
10 ³ - 10 ⁴	2 (7.4)	2 (6.5)
> 10 ⁴	2 (7.4)	5 (16)

There were paired caecal samples for 772 of the carcass swabs. Of these, three were positive only on the carcass and 70 were positive only in the caecum ([Table 4](#)). One lamb was positive for *S. enterica* subsp. *diarizonae* serovar 61:k:1,5,7 in both sample types. The caecal levels for this lamb were 9.6×10^3 cfu/g (the levels in the corresponding swab sample could not be determined due to insufficient material).

Table 4. Numbers of caecal and carcass samples with detectable *Salmonella*. (NB - caecal data taken from Defra project OZ0348 for comparison.) Results for all samples tested and the subset of matched samples are given.

Sample type	Total number tested	Number positive for <i>Salmonella</i>	Percentage positive for <i>Salmonella</i>
Caeca (all)	1228	104	8.47
Swabs (all)	1282	7	0.55
Both caeca & swab (matched set)	772	1	0.13
Only caeca (matched set)	-	70	9.07
Only swab (matched set)	-	3	0.39

5.3. *Campylobacter* isolation and enumeration

Over the year, a total of 366 ovine carcass swabs (297 lamb, 65 ewe, 4 unspecified) were tested for *Campylobacter* of which 76 had detectable levels (20.8%). In comparison, 194/411 (47.2%) caecal samples were positive (data from the associated PATH-SAFE project). There was variation over the course of the year in the rates of carcass contamination, ranging from 2.9% to 63%, with lowest levels seen in the winter months (although only two samples were taken in February, in the final week) and a peak in late summer/early autumn ([Table 5](#); [Figure 2](#)).

The majority of isolates were *C. jejuni* (n=73; 95%) with the rest being *C. coli* (n=3) and *C. fetus* (n=1). One carcass was positive for both *C. jejuni* and *C. coli* (hence 77 isolates from 76 positive swabs). The prevalence rate on carcasses of sheep under-12-months was slightly lower than those over-12-months (15.4% vs 21.6%), although considerably fewer samples were tested from the former group (65 vs 296, with 4 'age not specified'). The difference was not statistically significant ($p=0.223$, χ^2).

Table 5. The numbers of carcass swabs tested each month (February 2023 to January 2024) for *Campylobacter* and the numbers that were positive. NB - February and March have been combined as only two samples were taken in February (both in the final week).

Sampling period	Number of tests	Number of <i>Campylobacter</i> positives (%)
Feb/Mar 2023	29	5 (17.2)
Apr 2023	39	10 (25.6)
May 2023	30	6 (16.7)
Jun 2023	43	6 (14)

Sampling period	Number of tests	Number of <i>Campylobacter</i> positives (%)
Jul 2023	21	5 (19)
Aug 2023	19	12 (63)
Sept 2023	39	12 (30.1)
Oct 2023	35	8 (22.9)
Nov 2023	42	9 (21.4)
Dec 2023	34	2 (5.9)
Jan 2024	35	1 (2.9)
Total	366	76 (20.8)

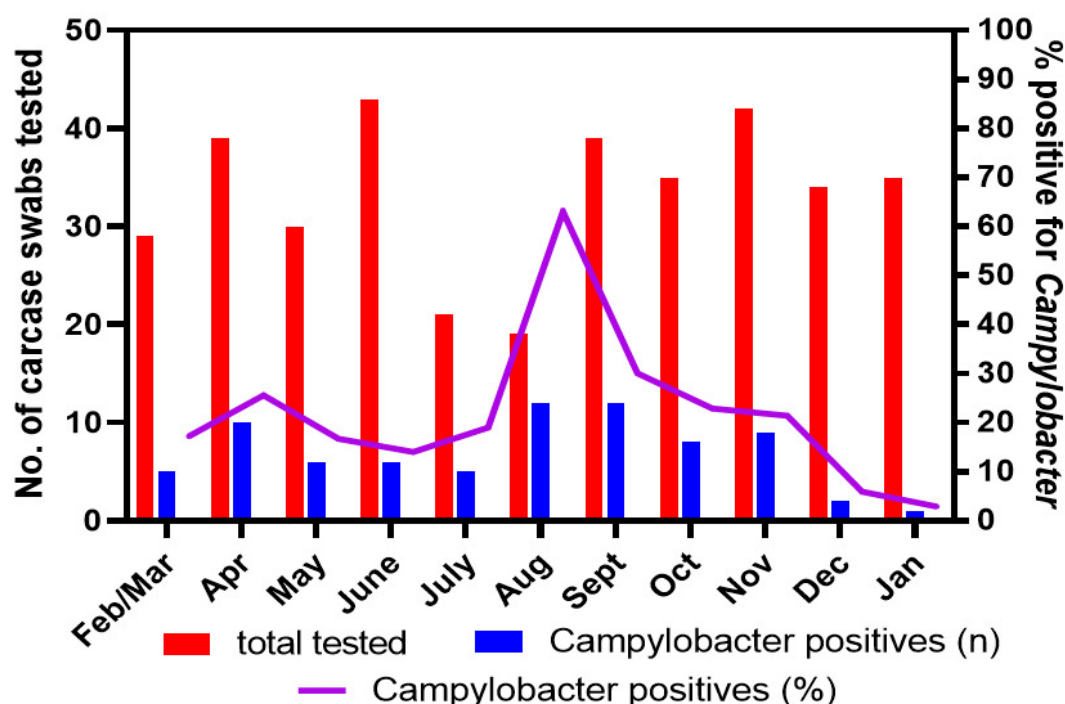


Figure 2. The numbers of carcass swabs tested each month (February 2023 to January 2024) for *Campylobacter* and the numbers that were positive. NB – February and March have been combined as only two samples were taken in February (both in the final week).

The levels of *Campylobacter* contamination were quantified by a direct plating method. The majority of positive samples had levels below the direct detection limit (< 40 cfu/swab; 57/75, 76%). (Table 6). Only two carcasses, both lambs, had levels of 10^4 cfu/swab or higher (1.2×10^4 and 2×10^5 cfu/swab).

Table 6. Levels of *Campylobacter* in lamb and ewe carcass swab samples (cfu/swab). Numbers of carcasses falling within specified ranges of surface contamination are shown.

Contamination level (cfu/swab)	Number of lamb carcasses (%)	Number of ewe carcasses (%)	Number of carcasses of unknown age	Total (%)
ND*	232 (78)	55 (85)	3	290 (79)
< 40	50 (17)	7 (11)	1	58 (16)
40-90	5 (1.7)	1 (1.5)	-	6 (1.6)
$1 \times 10^2 - 6 \times 10^2$	4 (1.3)	2 (3)	-	6 (1.6)
$1 \times 10^3 - 4 \times 10^3$	4 (1.3)	-	-	4 (1)

Contamination level (cfu/swab)	Number of lamb carcasses (%)	Number of ewe carcasses (%)	Number of carcasses of unknown age	Total (%)
1×10^4	1 (0.34)	-	-	1 (0.27)
2×10^5	1 (0.34)	-	-	1 (0.27)

*ND – none detected

There were paired caecal samples for 112 of the carcase swabs. Of these, 32 were positive only on the carcase, 47 were positive only in the caecum, and 16 were positive in both samples ([Table 7](#)).

Table 7. Numbers of caecal and carcase samples with detectable *Campylobacter*. (NB - caecal data taken from PATH-SAFE project OG0281 for comparison.) Results for all samples tested and the subset of matched samples are given.

Sample type	Total number tested	Number positive for <i>Campylobacter</i>	Percentage positive for <i>Campylobacter</i>
Caeca (all)	412	193	46.8
Swabs (all)	366	74	20.2
Both caeca & swab (matched set)	112	16	14.3
Only caeca (matched set)	112	47	42
Only swab (matched set)	112	32	28.6

5.4. Enterobacteriaceae and *E. coli* enumeration

One hundred and three carcase samples (81 lamb, 17 ewe and 5 age unknown) were randomly selected to determine levels of microbial contamination, using *Enterobacteriaceae* and *E. coli* as indicator organisms. Different methodologies with differing detection limits were used for each. For *E. coli* the MPN method had a quantifiable range of 2.7×10^1 – 1.4×10^4 cfu/swab. For *Enterobacteriaceae* the direct plate count method had a quantifiable range of 4×10^1 – 1.3×10^6 cfu/swab. For *Enterobacteriaceae* 77/103 samples (75%) had detectable (74% and 76% of lamb and carcase samples respectively) ([Table 8](#)). For *E. coli* 91/103 (88.4 %) samples had detectable levels (89% and 88.2% of lamb and carcase samples respectively) ([Table 9](#)). There was no significant difference in rates and levels of contamination between lamb and ewe carcase samples ($p=0.591$ and $p=0.703$ for *Enterobacteriaceae* and *E. coli* respectively). From all swabs tested 40% and 32% of samples had low levels ($<10^2$ cfu/swab) of *Enterobacteriaceae* and *E. coli* respectively, 44.6% and 49.5% had medium levels (10^2 - 10^4 cfu/swab), 15.5% and 18.4% had high levels ($>10^4$ cfu/swab). Six lamb (7.4%) and one ewe carcase swabs (5.9%) had no detectable levels of either.

Table 8. Levels of carcase contamination with *Enterobacteriaceae*. Numbers of samples falling within specific ranges of microbial load (cfu/swab) are given.

Sheep age	Swabs tested	Swabs positive	<10 ² cfu/swab	10 ² -10 ³ cfu/swab	10 ³ -10 ⁴ cfu/swab	10 ⁴ -10 ⁵ cfu/swab	10 ⁵ -10 ⁶ cfu/swab	>10 ⁶ cfu/swab
<12 months	81	60	13	21	12	11	1	2
>12 months	17	13	1	9	2	0	1	0
NK*	5	4	1	1	1	1	0	0
Total	103	77	15	31	15	12	2	2

*NK – not known

Table 9. Levels of carcase contamination with *E. coli*. Numbers of samples falling within specific ranges of microbial load (cfu/swab) are given.

Sheep age	Swabs tested	Swabs positive	<10 ² cfu/swab	10 ² -10 ³ cfu/swab	10 ³ -10 ⁴ cfu/swab	>10 ⁴ cfu/swab
<12 months	81	72	17	18	21	16
>12 months	17	15	3	4	6	2
NK*	5	4	1	0	1	2
Total	103	91	21	22	28	20

*NK – not known

6. Discussion

This study, in conjunction with the associated government funded caecal carriage studies is the largest scale investigation in 20 years on the bacteriological status of sheep at slaughter in England and Wales. The 12 slaughterhouses that took part represented approximately 28% of the annual national sheep throughput in England and Wales. Although good geographical representation was achieved, it is possible that a larger cohort would have allowed comparison of different practices within the abattoir industry which was not possible here.

Our study investigated the levels of pathogenic *Salmonella* and *Campylobacter* and non-pathogenic bacteria *E. coli* and *Enterobacteriaceae* on the surfaces of cull ewe and lamb carcasses, along with quantification of caecal levels of *Salmonella*. Some level of carcase surface contamination by intestinal bacteria is an almost inevitable consequence of slaughter and evisceration processes (Dickson & Anderson, 1992). However, care is taken, and measures are in place to minimise contamination. The headline figures from our survey indicate

very low levels of carcase contamination with *Salmonella*, with only 7/1282 (0.55%) positive swab samples, although *Campylobacter* contamination was more common, being detected in 76/366 (20.8%) swab samples.

The last large scale abattoir survey, undertaken in 2003, focussed on pathogens in food animals (cattle, sheep and pigs) at slaughter, but only looked at intestinal carriage rather than carcase contamination (Milnes et al., 2008). Similarly, a national survey in 1999 also only looked at intestinal carriage of *Salmonella* (Davies et al., 2004). In these earlier surveys, estimates of intestinal carriage rates for *Salmonella* were 0.1% (1999) and 1.1% (2003). The Defra-funded intestinal carriage survey run in parallel to our carcase survey reported a prevalence of 8.39%. However, there are notable differences between the methodologies, in particular sample size and type: 1g of rectal faeces was used for the earlier studies compared to 10g of caecal contents used in the current one. Intestinal carriage of microorganisms is presumably a significant factor in the types and levels of carcase surface contamination so an increase in intestinal prevalence is a potential cause for concern. However, the low prevalence of *Salmonella* (0.55%) suggests the hygiene measures in place in the participating abattoirs are largely effective at minimising contamination by this organism.

Carcase contamination (along with abattoir environmental contamination) has been reported in a previous UK study (Small et al., 2006). This study was on a much smaller scale to ours, involving single visits to three abattoirs and swab sampling 240 lamb carcasses. A higher prevalence than our finding was reported (9.5%) although there was considerable variation between the abattoirs (0/120, 10/80, and 13/40 positive samples). No intestinal samples were collected, or quantitative bacteriology undertaken in this earlier study, so it is not possible to determine any difference between intestinal carriage and carcase contamination. However, the large, 16-fold difference between caecal carriage and carcase contamination by *Salmonella* we observed (i.e. 8.39% vs 0.55%) was not seen in a similarly large-scale study of pigs at slaughter. Across 14 UK abattoirs in 2013 (Powell et al., 2016) a *Salmonella* prevalence of 9.6% prevalence was reported for pig carcase contamination, but the caecal prevalence was only three-fold higher (30.5%). The differences in the findings of our study and the pig abattoir study are likely due to several factors, including abattoir processes and bacterial loads, and warrant further investigation. A low prevalence of *Salmonella* contamination (1.3%) was reported in a study from Australia which sampled 164 pre-chill ovine carcasses (Duffy et al., 2010). Nearly all the animals sampled were reported as being hoggets, i.e. sheep one to two years-of-age. In our study, the abattoirs only classified animals as less than or more than 12 months old so it is unknown how many of the latter would be classed as hoggets. Nevertheless, the prevalence of *Salmonella* contamination on carcasses of

sheep older than 12 months (1.6%) is very similar to that reported in the Australian study. Most samples in our study were from lamb carcasses (reflecting the relative numbers of ovine age groups presented for slaughter) where the prevalence was found to be four-fold less (0.4%), although the difference between the two age groups was not significant. With few comparable data from sheep in England and Wales, identification of any trends in *Salmonella* contamination is not possible.

Campylobacter contamination (76/366; 20.8%) was notably more prevalent than *Salmonella*: (7/1282; 0.55%). This is considerably lower than that found in a study from Scotland undertaken in 2004-05 (Garcia et al., 2010) which reported a 90% prevalence. However, the Scottish study was based on a much smaller sample number (n=80), all of which were collected from a single abattoir. A similarly high prevalence was also reported in a study on two Greek abattoirs sampled over an 18-month period. Prevalence of carcass contamination with *Campylobacter* was found to be 94.5% for lambs (2-5 months old) and 72.2% of sheep (4-6 years old) (Lazou et al., 2014). A difference between age groups was also seen in our study, although the age/prevalence relationship was reversed (albeit with different age criteria). We found prevalence was higher in sheep under 12 months old (22%) than over 12 months old (15%), but the difference was not significant. Interestingly, the Greek study also reported that intestinal carriage was considerably less frequent than surface contamination (34% vs 72.2%). This is the opposite to the findings of our study and the associated PATH-SAFE study (48% vs 20%) and suggests there was not only less contamination generally but also considerably less cross contamination of carcasses occurring in the English and Welsh abattoirs sampled in our study.

Not surprisingly, the relative carcass contamination rates for the *Salmonella* and *Campylobacter* are reflected to some extent in the caecal carriage rates determined in the two surveys run alongside this one: 8.39% for *Salmonella* and 48% for *Campylobacter*. The differences in contamination rates possibly reflect the overall numbers of bacteria carried by animals, although the data to confirm this is lacking. Caecal levels of *Salmonella* were mostly below 10^3 cfu/g (48/60) but similar data were not determined for *Campylobacter* here. Intestinal levels of over 10^7 cfu/g have been reported in an earlier UK study of *Campylobacter* in lambs at slaughter although these levels were in small intestine samples (Stanley et al., 1998).

The contamination levels of *Salmonella* and *Campylobacter* on carcasses were quantified as part of our study. As well as a higher prevalence, higher bacterial loads were noted for *Campylobacter* (20.8% positive, with up to 10^5 cfu per swab) compared to *Salmonella* (up to 10^3 cfu per swab) but with so few positives of the latter meaningful comparison is not possible.

Regarding *Salmonella* tests, 772 of the swab samples had matching caecal samples. The small number of *Salmonella* positives (4/772) in the matched samples also prevents detailed analysis. However, the fact that three of the four positive carcass swabs did not have detectable levels in caecal samples suggests that *Salmonella* contamination may have come from other sources, such as environmental contamination or cross contamination from other carcasses. There is some further evidence for this in the matched sample data for *Campylobacter*. Since there was a higher prevalence than *Salmonella*, there were more positive carcass swabs in the matched sample subset despite there being fewer pairs tested (48/112). Only 16/48 (33%) *Campylobacter* positive carcass swabs had positive, matched caecal samples, meaning two thirds of the carcasses were contaminated despite having no caecal carriage. Future WGS analysis of the *Salmonella* and *Campylobacter* isolates from the matched samples (and strains from unmatched samples) should shed some light on the sources of contamination.

Levels of *Enterobacteriaceae*, which includes *E. coli*, are used as indicators of hygiene standards in the food industry and sampling of carcasses and meats occurs regularly through the implementation of the meat hygiene legislation (Commission Regulation [EC] No 2073/2005 on Microbiological Criteria for Foodstuffs, as adopted in UK legislation). The FSA does not have sight of the results of the testing carried out by food business operators. Our survey was designed for surveillance purposes as opposed to examining legislative limits. We determined levels of *Enterobacteriaceae* and *E. coli* on the surface of the sheep carcasses with results expressed as the number of bacteria per swab sample. Prevalence of these indicator organisms was high, which was as expected for freshly slaughtered meat. For *E. coli* 91/103 (88.4 %) samples had detectable levels (89% and 88.2% of lamb and ewe carcass samples respectively) and 77/103 samples (75%) had detectable *Enterobacteriaceae* (74% and 76% of lamb and carcass samples respectively). Seven carcasses (6.8%) had no detectable levels of either. There was no significant difference in rates and levels of contamination between lamb and ewe carcass samples. The *Enterobacteriaceae* and *E. coli* results were broadly similar: 40% and 32% of samples had low levels ($<10^2$ cfu) of *Enterobacteriaceae* and *E. coli* respectively, 44.6% and 49.5% had medium levels (10^2 - 10^4 cfu), 15.5% and 18.4% had high levels ($>10^4$ cfu). The rates of contamination are similar to those reported in a study of Norwegian sheep carcasses which found detectable levels of *Enterobacteriaceae* in 92% (229/250) and *E. coli* in 87% (218/250) of samples (Alvseike et al., 2019).

The *Enterobacteriaceae* family includes *E. coli* so in theory the level of the former should always be at least equal to the latter, but this was not always the case. There were only 11 samples (10.7%) with no detectable *E. coli* compared to 26 (25%) with no *Enterobacteriaceae*. However, different

methodologies were used for each. The *E. coli* MPN method had a lower limit of detection (27 cfu/swab) than the direct plating method used for Enterobacteriaceae enumeration (40 cfu/swab): nine swabs had levels of *E. coli* between 27 and 40 cfu. A higher incidence of *E. coli* than Enterobacteriaceae in some samples was also reported in the Norwegian study (Alvseike et al., 2019).

The microbiological quality of fresh meat is a public health concern due to the potential for contamination with organisms able to cause foodborne illness in consumers. *Campylobacter* and non-typhoidal *Salmonella* are the two most common bacterial agents of foodborne illness in the UK and elsewhere in the world. In the case of *Salmonella* there is great variation in the health risks associated with serotype. The serovars with most significance for public health are *S. enterica* subsp. *enterica* serotype Enteritidis and *S. Typhimurium* which accounted for 25.7% and 21.3% respectively of isolations from humans in UK in 2022 (DEFRA, 2023). Relatively few *Salmonella* outbreaks are attributed to ovine meat and products. In the 2018 and 2019 EU Zoonoses reports only 4/122 outbreaks were attributed to ovine meat and products. Nevertheless, ovine-attributed outbreaks do cause significant health problems. An outbreak of a strain of *S. Typhimurium* that caused clinical signs in sheep, including mortality, was linked by WGS to a human outbreak cluster (Carson & Davies, 2018). Sequence analysis determined human cases of this strain first occurred in 2017. By the end of 2018, almost 300 cases were confirmed, including one death ([Almost 300 sick and one dead due to Salmonella in UK | Food Safety News](#)). More recently, in 2021, 22 people at a barbecue became ill after consuming a lamb's liver dish contaminated with *S. Typhimurium* (Adamson et al., 2024). In our survey, two of the seven isolates from carcass swabs were *S. Typhimurium* (one of which was a monophasic variant) which is a potential health concern. The other five isolates were *S. enterica* subsp. *diarizonae* serotype 61:k:1:5 which is a host-adapted type associated with sheep and has no confirmed association with human disease (Rubira et al., 2021). These rates are broadly in line with the findings of the Defra's 2023 caecal survey in which 77% of 104 isolates were *S. diarizonae* serotype 61:k:1:5 and 21% were *S. Typhimurium*.

Human infections of *Campylobacter* have been linked to red meat, including lamb (Neimann et al., 2003; Raji et al., 2000). The majority of all human cases are due to *C. jejuni* (Gillespie et al., 2002) which was the species most commonly isolated in our study (96%), so the levels of contamination we observed potentially pose a greater health concern than the low levels of *Salmonella*. The vast majority of human *Campylobacter* cases are sporadic, and their sources are often not definitively determined. Relative risks of different food types are estimated from epidemiological risk studies and genotyping of isolates from different sources. A systematic

review of source attribution of human campylobacteriosis using multilocus sequence typing identified contaminated chicken products as the major risks. However, ruminant (cattle or sheep) sources were implicated in a substantial number of cases. The relative risks of cattle and sheep products are difficult to determine as the predominant sequence types (STs) are the same for both hosts, but these STs are associated with human cases (Cody et al., 2019). Further characterisation of sheep isolates is needed to fully assess the risks to human disease they pose. The risks presented by contaminated carcasses may diminish as they move to retail. Unlike *Salmonella*, *C. jejuni* and *C. coli* do not tolerate aerobic environments very well (Kelly, 2008). *Campylobacter* spp. are also reported to be susceptible to drying, and during normal chilling processes their levels may well decrease from abattoir to retail (Grau, 1988). Interestingly, in an FSA survey of red meat at retail in the UK in 2006-07, a low prevalence of *Campylobacter* contamination was reported: 7/798 (0.88%) in the English and Welsh samples (FSA Project B18018). The survey also reported 0% prevalence of *Salmonella* and 23.5% prevalence of *E. coli* contamination in the same samples. Although the retail survey and ours are not directly comparable, the results of the former do not rule out the possibility that the bacterial levels we reported may fall further along the food processing chain. Further exploration of this would be worthwhile.

7. Concluding remarks

This study has produced a valuable data set giving a good indication of the microbial quality of lambs and ewes at slaughter in England and Wales. The 12 slaughterhouses that took part in the study represented approximately 28% of the annual national sheep throughput in England and Wales. Although good geographical representation was achieved, a larger cohort may have allowed comparison of different practices within the abattoir industry which was not possible here. Encouragingly, despite relatively high prevalence of caecal carriage, levels of carcass contamination with *Salmonella* are very low. Furthermore, despite the prevalence of *Campylobacter* carcass contamination being higher there was still a notably lower prevalence compared to that in caeca. This suggests that the slaughtering, hide removal and evisceration processes are effective in preventing extensive carcass contamination with pathogenic *Salmonella* and *Campylobacter*. Evidence for hygienic practices is also provided by the low numbers of indicator bacteria found on most carcasses sampled. High numbers of *Campylobacter* and indicator organisms on some carcasses suggest there is potential for improved practices at slaughter. However, correct storage from slaughter to retail and cooking would be expected to reduce or eliminate any surface microbiological contamination that may be present.

These results provide valuable baseline data that will help in risk assessments/modelling and will help to inform future Government policy. The overall results will be disseminated to relevant parties in the meat industry and individual data sets will also be fed back to the participating abattoirs for them to assess their practices and act accordingly.

The survey has confirmed the presence of pathogenic *Salmonella* and *Campylobacter* on the surface of lamb and ewe carcasses albeit at low levels. It is important to follow the FSA's cooking advice and good hygienic practices when handling and preparing these meats within the kitchen so as to minimise consumer exposure to these pathogens.



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Supplementary Materials

Sheep survey appendices

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