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Microbial Quality of Australian Offal

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Executive Summary

A national baseline study of offal hygiene was commissioned by Meat and Livestock Australia (MLA). Key objectives of the study were to:

1. Estimate the prevalence and quantitative levels of indicator microorganisms on offal;
2. Estimate the prevalence of pathogens of concern to key markets; and
3. Select and test an appropriate range of offal from a range of establishments to assist market access.

Seventeen plants contributed offal samples (n=1,756) as part of the study. Samples were analysed for APC, *E. coli* and coliforms, with selected samples analysed for *Clostridium perfringens*, coagulase positive *Staphylococcus*, *Salmonella* and Shiga toxigenic *E. coli* (*E. coli* O157 and STEC serotypes O26, O45, O103, O111, O121 and O145). Offal types and sample numbers were based on export statistics for 2016 to 2018.

The microbiological quality of offal varied between plants with average APC values ranging between 1.58 and 4.13 Log CFU/g. The average APC on all offal types varied with species with beef, sheep, lamb and goat offal having average APC levels of 3.25, 3.38, 3.70 and 2.97 Log CFU/g, respectively. There was little difference in APC on offal sampled frozen (3.26 Log/CFU/g) and that sampled fresh (3.73 Log CFU/g). While the study was not designed to compare between offal types there was considerable variability in APC on offal.

Salmonella was detected in 12 of 627 samples analysed (1.9%). Isolation rates from beef and goat samples were about the same (~2.2%), while *Salmonella* was not isolated from lamb and sheep offal. Beef head meat was the offal most often associated with *Salmonella* detections (5/87 or 5.7%).

Beef offal classified as raw ground beef components returned three STEC detections (1.11%) and 2 positive *E. coli* O157 detections (0.74%). Both *E. coli* O157 isolates were obtained from head meat while STEC isolates were associated with head meat (n=1) and heart (n=2).

The significance of these results is discussed with reference to the published literature. While the microbiological quality of offal varied between offal type and processing establishment and while the APC and prevalence of *E. coli* were higher than typically found on meat, the average levels could be considered acceptable and consistent or better than those reported for similar products in the literature.

It is recommended that further investigations into possible sources and interventions for pathogen contamination of RGBC offal be undertaken.

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1 Background

The Harris report (2018) on non-tariff barriers to trade, identified offal as a high priority for the Australian meat industry, with an estimated worth of $363m. For Australia to address possible trade issues and to open new markets, the hygienic quality of offal needed to be assessed and documented. A national baseline study of offal hygiene was commissioned by Meat and Livestock Australia (MLA).

Key objectives of the study were to:

1. Estimate the prevalence and quantitative levels of indicator microorganisms on offal;
2. Estimate the prevalence of pathogens of concern to key markets; and
3. Select and test an appropriate range of offal from a range of establishments to assist market access.

In order to meet the study objectives a selection of export plants was invited to participate in the survey. Of the 17 plants that agreed to participate, 10 processed beef, 5 processed lamb, 6 processed sheep and 3 processed goats (some plants processed multiple species). Offal samples (n=1,756) from these plants were collected by plant staff and sent to the ISO 17025/NATA accredited testing laboratory for analysis. Samples were analysed for numbers of indicator bacteria i.e. aerobic plate count (APC), *E. coli* and coliforms. Selected samples were quantified for potential pathogens i.e. *Clostridium perfringens* and coagulase positive *Staphylococcus*. The prevalence of *Salmonella* was estimated in 627 offal samples. Beef offal (n=270) classified as a raw ground beef component was analysed for *E. coli* O157 and Shiga toxigenic *E. coli* of serotypes O26, O45, O103, O111, O121 and O145. An analysis of the national baseline data for all participants is provided in this report.

2 Methodology

2.1 Sampling

Sampling was undertaken by establishment personnel from frozen or fresh product.

A minimum of 50 g of sample was collected from the surface of frozen cartons except in the case of RGBC products sampled at beef establishments where a minimum of 400g was collected from the surface of each carton selected for sampling. For chilled product, an individual piece (weighing at least 50g or 400g in the case of RGBC if applicable) of each offal type sampled was placed in a separate bag.

RGBC samples were collected using the same protocols as outlined in DAWR MN 2010/03 (or the Microbiological Manual for Sampling and Testing of Export Meat and Meat Products, where it replaced by Meat Notice) relating to E. coli O157 testing of RGBC.

All samples were individually bagged and labelled with the date collected, the Establishment number, the offal type sampled, and other details required for the project. RGBC samples were labelled RGBC as well as the individual offal type e.g. cheek meat, weasand meat.

2.2 Transport methods

All samples were shipped refrigerated (≤ 7°C) by overnight courier to the testing laboratory such that the sample arrives at the testing laboratory no later than on the day after the samples were collected (or dispatched in the case of frozen samples). Fresh samples or samples that thawed during transport were not re-frozen.

2.3 Tests conducted

The tests for each sample were:

- RGBC - APC, E. coli / coliform, Salmonella (beef only), STEC (beef only)
- Other - APC, E. coli / coliforms, Coagulase positive staphylococci, Clostridium perfringens

Samples were tested at a NATA accredited and DAWR approved laboratory using the following methods.

- APC - AOAC 990.12 (Petrifilm)
- E. coli/coliform - AOAC 991.14 or AOAC 998.08 (Petrifilm)
- Salmonella - FSIS MLG 4C (BAX) (Confirmation of screen positives by Australian Standard method and serotpyed by Qld Health)
- STEC - FSIS MLG 5A and 5B (BAX) (Full confirmation on any screen positives samples)
- Coagulase positive staphylococci by Petrifilm method
- Clostridium perfringens by pour plate

Frozen samples were thawed in the laboratory at 18- 27°C for up to 3 hours before commencing the test (Australian Standard AS 5013.11.2-2006). Some samples also partly thawed during transport.

APC and E. coli/coliform samples consisted of a minimum of 25g of sample homogenised in 9x the weight (i.e. 1/10 referred to as -1 dilution) of peptone salt solution (or similar approved diluent). Sufficient dilutions were prepared to ensure that a count was obtained for every sample. Counts outside the countable range on Petrifilm were estimated where possible i.e. to avoid reporting TNTC results.

RGBC samples were analysed for APC and E. coli/coliforms as outlined above as well as for Salmonella using the FSIS MLG BAX protocol using a 375g sample. Salmonella detections were confirmed by the Australian Standard method and isolates forwarded to Qld Health Scientific Services for serotyping.

3 Data description

Count data was summarised in Excel™ using pivot tables. For the purpose of analysis, count data below the limit of detection (LOD) of the analytical method was assigned a value of ½ the LOD. Censoring of microbiological data is contentious and will bias results towards higher estimates of the mean, this is especially true when the number of samples containing enough organisms to count is low i.e. samples with a low apparent prevalence. Other methods may be more appropriate but require significant computational skills. Raw data including non-detects (ND) should be retained to allow for more accurate statistical analysis if deemed necessary. Count data was log transformed before analysis as is the convention with microbiological data.

Qualitative data obtained for pathogens was reported as the number of positive detections from the samples analysed. No account was made for the sensitivity of the analytical test or of the effect of sample size on the likelihood of detection.

Quantitative estimations (n=8,232) were made from 16 offal types collected from four species (beef, sheep, lamb and goat) processed at 17 meat export establishments. Sample numbers for each offal type and for each species were based on production volumes reported by MLA. A total of 1,756 samples were collected from beef (n=975), sheep (n=160), lamb (n=486) and goat (n=135) offal. The number of determinations carried out for each species is provided in Table 1.

Table 1: Number of quantitative estimations made from offal of beef, sheep, lamb and goat for each class of microorganisms.

Species	*C. perfringens*	Coliforms	*Staphylococcus* [a]	*E. coli*	APC	**Total**
Beef	701	975	701	975	975	**4327**
Sheep	160	160	160	160	160	**800**
Lamb	486	486	486	486	486	**2430**
Goat	135	135	135	135	135	**675**
Total	**1482**	**1756**	**1482**	**1756**	**1756**	**8232**

[a] Coagulase positive *Staphylococcus*

Offal types and sample numbers were based on export statistics for 2016 to 2018 (

Table 2).

Table 2: Sample numbers for offal types collected from each slaughter class and analysed for APC. Numbers and offal types based on export statistics for 2016-2018.

	Species			
Offal Type	Beef	Sheep	Lamb	Goat
Brain	-	2	25	-
Cheek	75	-	-	-
Head Meat	89	-	-	-
Heart	101	13	26	3
Kidney	60	20	133	23
Liver	107	32	110	2
Lungs	-	-	-	13
Pluck	-	-	-	14
Skirt	115	3	-	-
Spleen	-	-	-	16
Tail	102	-	-	-
Tendons	54	-	-	-
Testes	-	-	-	22
Tongue	103	10	107	-
Tripe	156	80	85	42

Weasand	13	-	-	-

Around 270 beef offal samples classified as raw ground beef components (RGBC; Cheek, Head Meat, Heart and Weasand) were analysed for the presence of Shiga toxigenic *Escherichia coli* (STEC, n=270) and *E. coli* O157:H7 (n=271). The distribution of samples among offal types analysed for *E. coli* O157:H7 are given as an example in

Table 3.

Table 3: Distribution of RGBC types samples analysed for *E. coli* O157:H7

RGBC	Sample No.
Cheek	74
Head Meat	87
Heart	98
Weasand	12

The presence of *Salmonella* was determined in 627 samples collected from all four species across all offal types.

4 Data Analysis

Data was visualised using R (2018). Where appropriate, statistical analysis of the data was performed using R or Minitab™ (Minitab 14, State College, PA). A level of significance of $p<0.05$ was assumed for all tests of significance. Count data was analysed using the 'aov' function in R while prevalence estimates were tested for association using the Chi-Squared function in Minitab™.

4.1 Seasonal Variation

Samples were collected through September 2018 to June 2019. The time distribution of APC results for all beef samples is shown in Fig. 1. Sampling was clearly biased towards the later part of 2018. This makes it difficult to draw any conclusions about seasonal effect on the hygiene of offal.

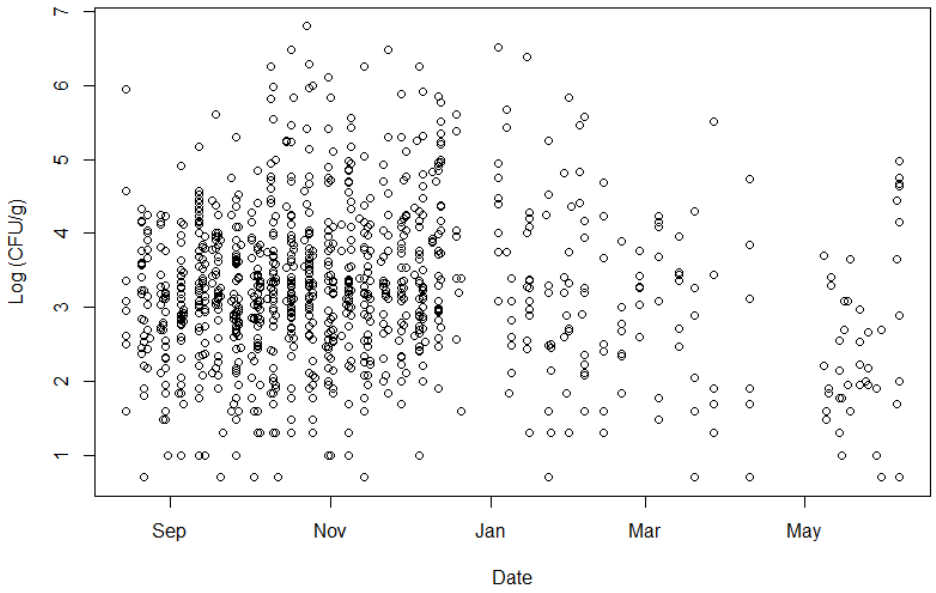

Fig. 1: Time distribution of APC data for beef offal

There is a suggestion that monthly mean APC on beef offal falls during the cooler months and increases in summer (Fig. 2). But again, given the distribution of samples over time it would not be recommended to draw any conclusions from this. Data for other quantified microorganisms was not analysed for time effects because of the clustering of samples in 2018.

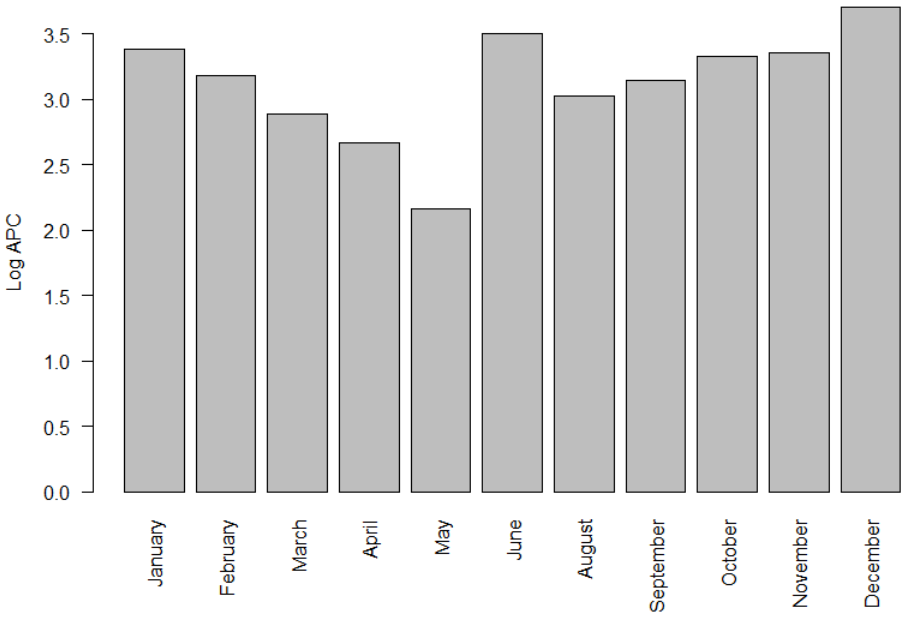

Fig. 2: Distribution of monthly mean APC on beef offal over time. Note that the x-axis is not in chronological order in that months January through June were in 2019 while months August through December were in 2018.

4.2 Effect of Establishment

Microbiological quality varied between plants with average APC values ranging between 1.58 and 4.13 Log CFU/g (Fig. 3). It is not clear if this is due to different ratios of offal types sampled at each establishment or to inherent differences in processing. As plants were self-selecting it is not clear if this variability is typical of the entire industry.

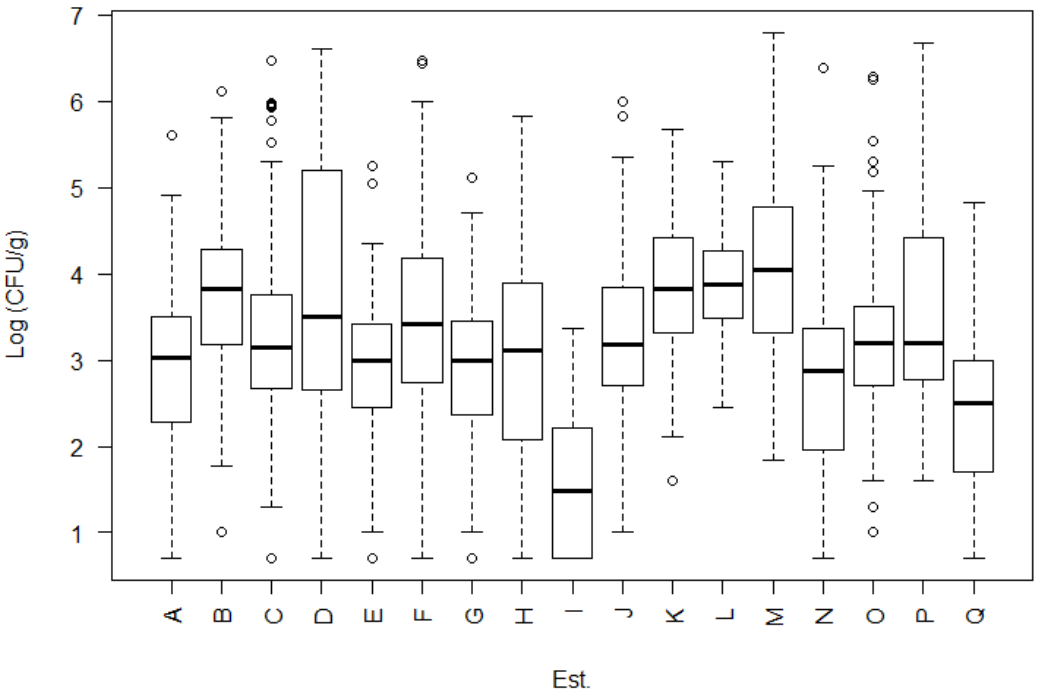

Fig. 3: Variability in APC from offal products produced at all participating establishments.

4.3 Type of Refrigeration

Samples were collected from either fresh or frozen product. There was little difference in Log APC values between the two refrigeration types as shown in Fig. 44; and because of this refrigeration type was excluded from further analysis.

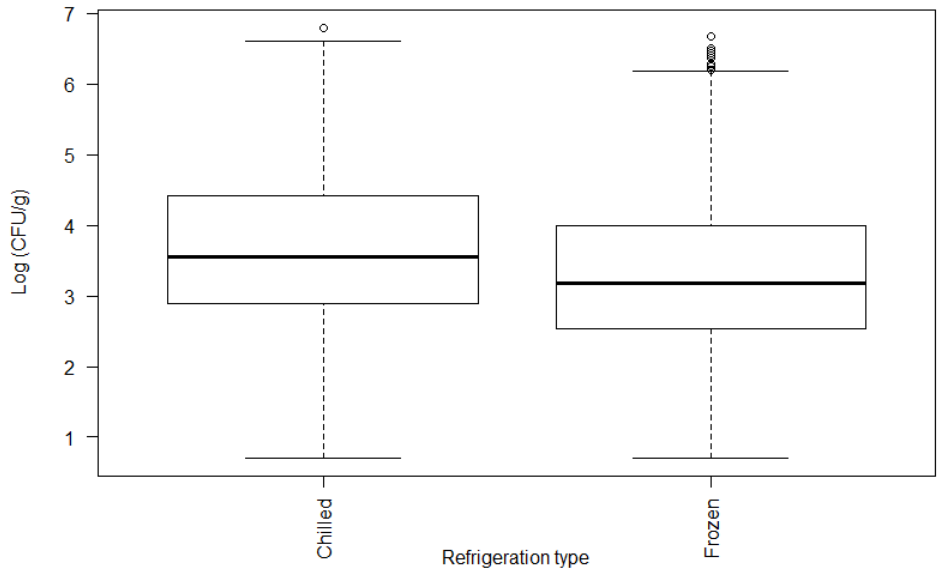

Fig. 4: Box plot of log of the APC for all offal samples collected either chilled or frozen

4.4 Species Comparison

There were slight differences in the APC found on offal between species (Fig. 55). It should be noted that the survey was not designed to allow for a comparison to be made between species, therefore the results of any comparison should be interpreted with caution. Nevertheless, there were significant differences between species in relation to the overall level of bacteria found on offal samples (Table 4). The differences noted would not be considered biologically significant and are probably within the expected experimental error of the procedure, with the possible exception of the observed difference between goat and lamb APC.

Table 4: Average Log APC found on beef, goat, lamb and sheep offal samples. Values in columns with the same letter are not significantly different.

Species	Average Log APC
Beef	3.25[a]
Goat	2.97
Lamb	3.70
Sheep	3.38[a]

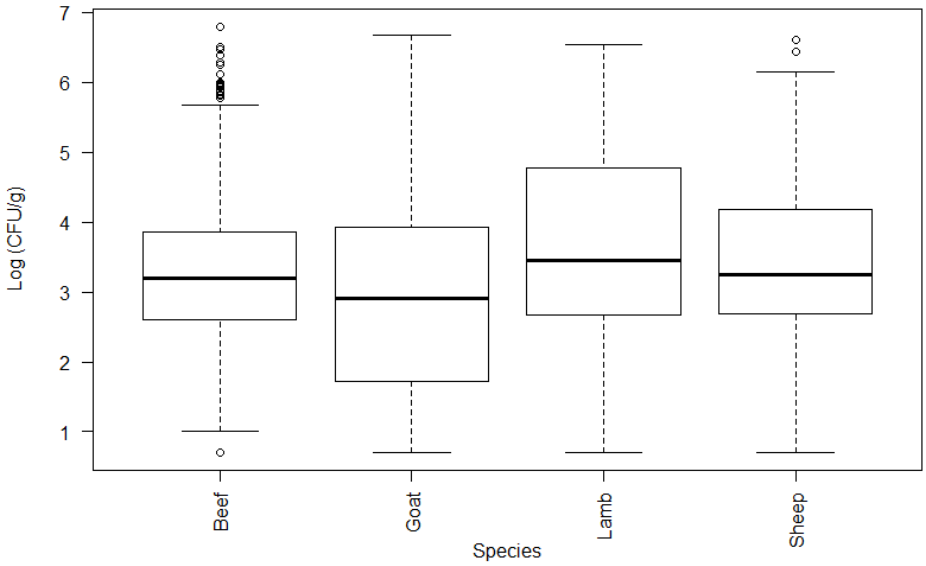

Fig. 5: Box plot of log APC for all offal samples collected from beef, goat, lamb and sheep.

4.5 Indicator Bacteria

Quantitative data obtained for indicator bacteria is summarised in

Table 5. In order to estimate the average level of target bacteria in each sample the count from samples in which the target bacteria was not detected was assigned a value equal to ½ the limit of detection (LOD) of the test method. Censoring of data in this way will result in an overestimation of the mean count as it ignores the possibility of truly negative samples.

Statistical analysis of the data obtained for *C. perfringens* was not carried out as the prevalence of this organisms on offal samples from all species was low. Coagulase positive *Staphylococcus* were isolated from between 3.7% and 11.9% of offal from individual species. The significance of differences in the prevalence of indicator bacteria is shown in

Table 5.

Table 5: Sample numbers and observed prevalence and average log count of indicator bacteria quantified from beef, goat, lamb and sheep offal samples. Average log count data was censored by assigning a value of ½ the LOD to samples with counts below the limit of detection of the method i.e. <10 CFU/g. Values, in columns, with the same letter are not significantly different.

Test/Species	Sample No.	No. positive	Prevalence			Average Log Count
C. perfringens [1]						
Beef	701	4	0.6%			0.707504
Goat	135		0.0%			0.69897 [2]
Lamb	486	3	0.6%			0.700828
Sheep	160	4	2.5%			0.711359
Coliforms						
Beef	975	297	30.5%	a		0.955884
Goat	135	58	43.0%		b	1.277667
Lamb	486	182	37.4%		b	1.060828
Sheep	160	61	38.1% [3]	a	b	1.060995
Staphylococcus						
Beef	701	73	10.4%	a		0.778031
Goat	135	5	3.7%		b	0.731285
Lamb	486	58	11.9%	a		0.816017
Sheep	160	6	3.8%		b	0.731274
E. coli						
Beef	975	150	15.4%	a		0.810189
Goat	135	53	39.3%			1.140444
Lamb	486	85	17.5%	a		0.828104
Sheep	160	45	28.1%			0.93084
APC						
Beef	975	965	99.0%			3.250014
Goat	135	116	85.9%			2.965672
Lamb	486	481	99.0%			3.697819
Sheep	160	158	98.8%			3.382554

[1] No statistical analysis undertaken as expected values were <5 for all cells

[2] ½ LOD of the method indicating that no organisms were detected

[3] Not significantly higher than beef, p=0.053

4.6 Microbial Quality of Different Offal Types

There was considerable variability in the aerobic plat count (APC) between offal types (Appendix 1). For example, on beef offal samples the liver was the least contaminated, while Tongue samples had the highest APC (Fig. 66).

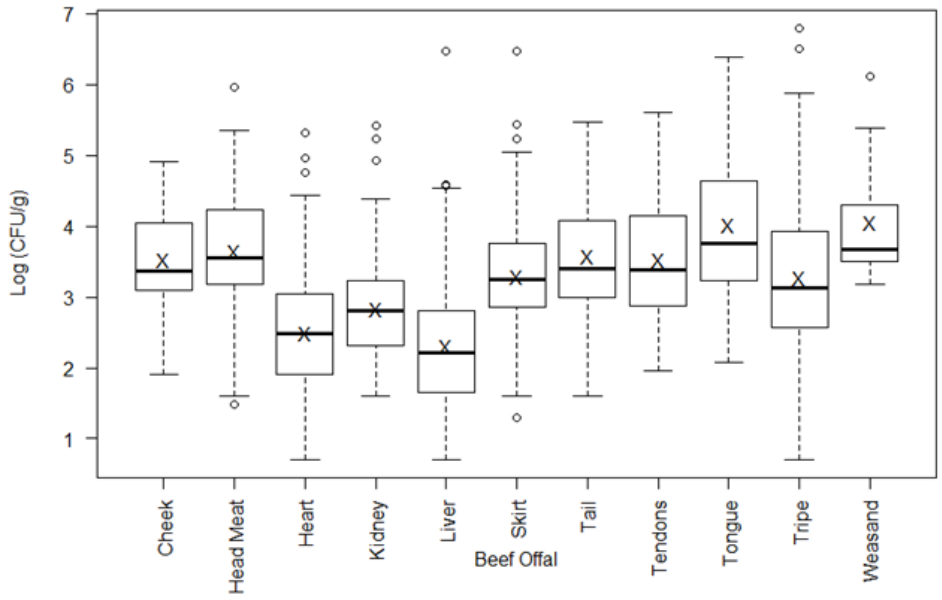

Fig. 6: Box plot of the log APC for beef offal samples

For some sheep and goat offal types only a small number of samples were analysed. These offal types could not be meaningfully included in the analysis and were combined under the heading 'Other'. This included brain, heart, skirt and tongue for sheep and heart, liver and spleen data for goat. Further, lung and pluck data for goat were combined under the heading pluck. Average log APC values and sample numbers for offal types for each species are summarised in Table 6,

Table 7, Table 8 and Table 9.

Table 6: Average Log APC for different beef offal types and number of samples analysed. Log APCs that have the same letter are not significantly different.

Offal Type	Log APC				Sample No.
Liver	2.30		b		107
Heart	2.50		b	c	101
Kidney	2.83			c	60
Tripe	3.28	a			156
Skirt	3.29	a			115
Cheek	3.52	a			75
Tendons	3.53	a			54
Tail	3.57	a			102
Head Meat	3.65	a		d	89
Tongue	4.01			d	103
Weasand	4.06	a		d	13

Table 7: Average Log APC for different lamb offal types and number of samples analysed. Log APCs that have the same letter are not significantly different.

Offal Type	Log APC		Sample No.
Kidney	2.80	a	133
Heart	2.83	a	26
Liver	2.86	a	110
Brain	3.55		25
Tongue	5.26		107
Tripe	4.54		85

Table 8: Average Log APC for different sheep offal types and number of samples analysed. Log APCs that have the same letter are not significantly different.

Offal Type	Log APC			Sample No.
Liver	2.81		b	32
Kidney	3.18	a	b	20
Tripe	3.46	a		80
Other	3.95	a		28

Table 9: Average Log APC for different goat offal types and number of samples analysed. Log APCs that have the same letter are not significantly different.

Offal Type	Log APC			Sample No.
Plucks	1.83		b	27
Kidney	2.73	a		23
Testes	3.05	a		22
Tripe	4.51			42
Other	1.51		b	21

Beef offal was consolidated into two groups, raw ground beef components (RGBC; includes cheek, heart, head meat and weasand) or other, so that the potential risk associated with the export of certain offal to the US could be determined. While the average Log APC on RGBC (3.20) was significantly lower than that on other offal (3.27), the difference was not meaningful from a practical perspective (Fig. 77). The observed prevalence of *E. coli* and coliform bacteria on RGBC and other offal was the same, 19% and 13.9%, respectively, while the count on RGBC and other beef offal was 0.83 and 0.80 Log CFU/g (censored data). The prevalence of coliform bacteria was also similar on RGBC (34.1%) and other beef offal (29%).

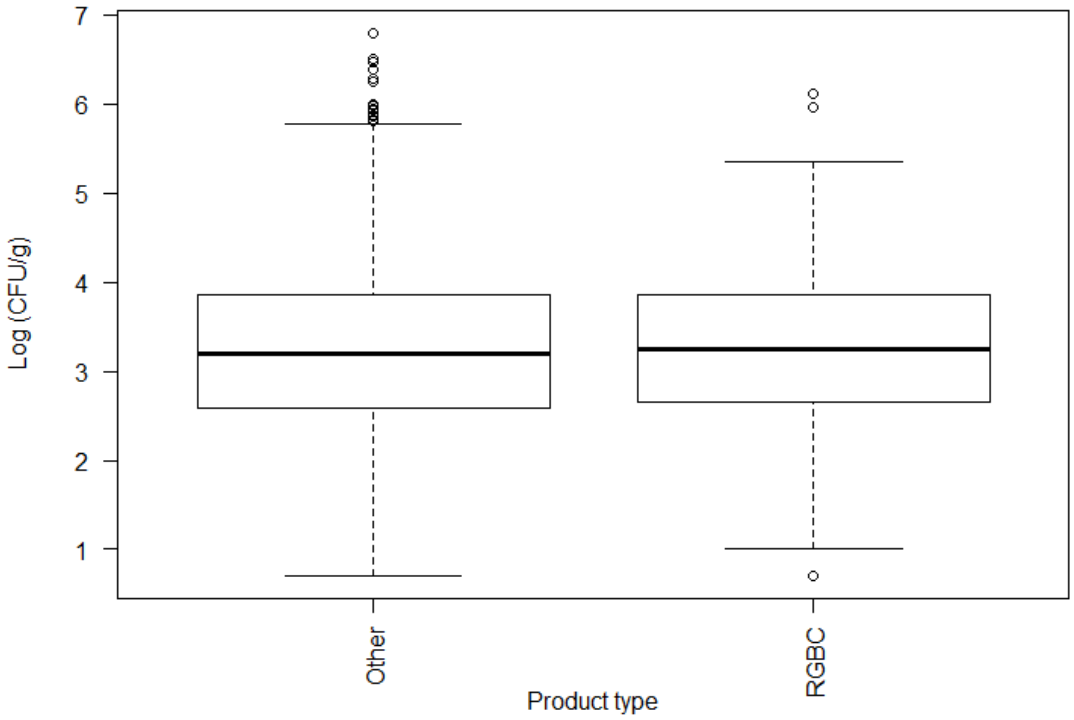

Fig. 7: Distribution of APC on RGBC and other beef offal.

5 Pathogens

5.1 *Salmonella*

Salmonella was detected in 12 of 627 offal samples analysed (1.9%). Isolation rates from beef and goat offal samples were about the same (2.3% and 2.2%, respectively). Interestingly, no *Salmonella* were found in lamb and sheep offal samples tested (0/100). This appears to go against the commonly held belief that sheep products are more frequently contaminated with Salmonella than beef products; however, only a relatively small number of sheep and lamb samples were analysed compared to beef and goats. The absence of Salmonella in sheep offal could not be assumed from this study. Beef head meat was the only offal type that had multiple detections for Salmonella (5/87 or 5.7%). This may have some implications if Salmonella testing of RGBC is implemented in the US. Salmonella detections were not evenly distributed between the participating establishments. Goat establishment 'P' accounted for all 3 of the Salmonella isolates recovered from goat offal; while plant 'C' accounted for 6 of the 9 Salmonella isolates found on beef offal and Salmonella was detected in 4 of 18 head meat samples (22%) from this establishment. The overall prevalence of *Salmonella* at plant 'C' was 11.3%.

Serotyping information was only available for 8 of the 12 *Salmonella* isolates. Types Dublin and Typhimurium were isolated on two occasions each, while Newport, Infantis, Anatum (var15+) and subspecies I (var 15) were isolated on one occasion each.

5.2 STEC Including E. coli O157

Offal types classified as raw ground beef components (RGBC) were tested for the presence of STEC serotypes (O26, O111, O103, O121, O145 and O45) and *E. coli* O157 using the BAX PCR platform. STEC were detected in 3 of 270 samples (1.11%) while *E. coli* O157 was detected in 2 of 271 samples (0.74%). Both *E. coli* O157 isolates were obtained from head meat while STEC isolates were associated with head meat (n=1) and heart (n=2). In all three cases the STEC serotype detected was O26. The virulence markers associated with the *E. coli* O157 isolates complied with the MLG method definitions.

Screen test positives (potential positives) were obtained more often for STEC screening (30.7%) than *E. coli* O157 screening (4.1%). The conversion rate of potential positives to presumptive positive (a suspect colony on a selective agar plate) was 45.5% for *E. coli* O157 and only 3.6% for STEC. Final confirmation rates (presumptive to confirmed) were 40% for *E. coli* O157 and 100% for STEC.

Screen positive results are costly to industry and can reflect poorly on Australian product irrespective of the final confirmation result. The distribution of STEC and *E. coli* O157 screen positive results for offal types is shown in

Table *10*.

Table 10: Distribution of STEC screen positive samples between RGBC offal types.

Offal Type	Sample No.	Screen Positive	Percent
E. coli O157			
Check	74	1	1.4%
Head Meat	87	9	10.3%
Heart	98	0	0.0%
Weasand	12	1	8.3%
STEC			
Check	74	28	37.8%
Head Meat	87	45	51.7%
Heart	97	3	3.1%
Weasand	12	7	58.3%

6 Discussion

The variability in count on different offal types would be expected to be high, due both to their anatomical location in the animal and to the type of processing. Internal organs such as the heart and liver would be essentially sterile at the time they are harvested, then potentially becoming contaminated during the evisceration process. Other offal such as the tripe and intestine are inherently contaminated due to their function in the animal. In the current study internal organs such as the kidneys, livers, hearts and goat pluck tended to have significantly lower counts than other offal types, in fact goat pluck had the lowest APC of any offal type tested.

The microbiological quality of offal has previously been considered generally of a lower standard than meat. While overall the APC found on offal in this study was higher than would be expected on bulk packed meat, the counts on kidneys, livers and hearts were similar. Currently, data on the hygienic quality of beef, sheep, lamb and goat meat is collected under the Department of Agriculture's Product Hygiene Indicators (PHI) program; however, this data is not publicly available. In the 2011 Australian national meat microbiological baseline study, the average APC on boneless beef was estimated to be 2.22 Log CFU/g with *E. coli* found in 2.1% of samples (Phillips et al, 2012), this compares with 3.25 Log CFU/g (ranging from 4.06 Log CFU/g in Weasand meat to 2.30 Log CFU/g in livers) and 15.4% for beef offal in the current study; and with 2.49 Log CFU/g and 7.8% for beef kidneys, livers and hearts. Similarly, bulk packed sheep meat in the 2011 baseline study had an APC and *E. coli* prevalence of 2.8 Log CFU/g and 12.5%, respectively (Phillips et al, 2013), compared with 3.62 Log CFU/g (from 5.28 Log CFU/g in tongue to 2.83 Log CFU/g in heart) and 20.1% for sheep (sheep and lamb data combined) offal in the current study; and with 2.85 Log CFU/g and 11.1% for kidneys, livers and hearts.

There are few published studies on the microbiological quality of offal. The APC on seven beef offal types prior to chilling at two New Zealand plants was reported to be in the range of 2.30 to 3.93 log CFU/cm^2 (Bell et al, 2000). *E. coli* prevalence in the same study ranged from 30% on kidneys to 100% on tripe, however as methods differ between that study and the current study it is not possible to compare prevalence estimates between the two studies. These counts were considered similar to counts reported on offal from the United States of America (Bell et al, 2000). Aerobic plate counts from the current study are compared to the New Zealand study in Table 11.

Table 11: Comparison of the hygienic status of beef offal in New Zealand (at packing; offal rinse Log CFU/ml) and in Australia (after chilling and/or freezing; excision sample Log CFU/g).

Offal Type	New Zealand [a]	Australia
Hearts	3.4	2.5
Kidneys	3.3	2.8
Livers	4.1	2.3
Tongues	3.7	4.0
Tails	3.4	3.6
Tripe [b]	3.8	3.3

[a] Adapted from Table 10 in Gill and Harrison (1985)

[b] Combined Seamy and mountain chain tripe

APC on Australian offal was similar to that reported on NZ offal prior to refrigeration (Table 11). It could be expected that chilling would have resulted in an increase in bacterial numbers meaning that bacterial numbers on Australian offal at a similar point in production may be lower than that reported in the NZ study.

All offal is harvested hot from the carcase and must be effectively cooled in order to prevent the growth of enteric pathogens such as toxigenic *E. coli* and *Salmonella*. Chilling has been shown to result in increases in *E. coli* on frozen sheep offal of between 0.42 and 2.26 Log CFU/ml (Gill and Harrison, 1985), with kidney, liver and heart showing the greatest increases; with 1.84, 2.07 and 2.26 Log CFU/ml, respectively. Beef kidney, liver and heart samples in the current study had the lowest prevalence of *E. coli* of any of the 11 offal types sampled. This may suggest that chilling of these offal is satisfactory. This pattern was not consistent with other species.

There was little difference in the current study between frozen and chilled samples. The APC on chilled samples was 3.73 compared with 3.26 Log CFU/g on frozen offal. Frozen samples had on average 0.1 log CFU/cm^2 less *E. coli* than chilled samples. This is in contrast to published data suggesting that freezing can result in decreases in *E. coli* numbers of between 0.5 to 0.9 log units compared to chilling alone (Gill and Harrison, 1985). Without knowing the individual chilling practices at each plant, it is not possible to comment on the likely reason for the lack of any observed reduction in *E. coli* numbers as a result of freezing.

Salmonella was isolated from 1.9% (12/612) of offal samples with 9 of the 12 positive samples coming from beef offal. Beef offal made up the bulk of the samples analysed. However, *Salmonella* was not frequently associated with beef kidney, liver and heart samples (1/128), suggesting low initial contamination levels on these offal or perhaps different harvesting or chilling practices. The majority (7/9) of the *Salmonella* detections in beef offal were associated with raw ground beef components, in particular head meat (5/82, 6.1%). Animals are known to mouth each other and objects in the holding area prior to slaughter. This may lead to contamination of saliva with *Salmonella* prior to slaughter and the subsequent contamination of head meat. Contamination of RGBC with *Salmonella* may be of concern if markets expand their focus on this product to include *Salmonella* and other pathogen testing. Interestingly no *Salmonella* were isolated from the 100 sheep and lamb samples analysed. Phillips et al (2013) found 17/551 frozen boneless sheep meat samples positive for *Salmonella* and none of 1,165 boneless beef samples (Phillips et al, 2012). These differences in *Salmonella* prevalence may be due to the low number of sheep and lamb offal samples or how these products are handled.

STEC including *E. coli* O157:H7 are found in red meats and would be expected to be found in offal. Asakura et al (2011) found 38/229 (16.6%) offal samples screen positive for STEC with 5 confirmed positives (2.1%), four *E. coli* O157:H7 and one O26 from intestine and omasum samples. Lee et al (2012) reported 4.2% STEC confirmed positive samples of omasum, abomasum and intestine; none of the 'big-six' serotypes or *E. coli* O157:H7 were found in that study. In the present study only RGBC offal types were examined for the presence of STEC. *E. coli* O157:H7 was isolated form 0.74% of RGBC offal samples (2/271) while STEC was isolated form 0.92% (3/270) of samples. The prevalence of STEC on offal appears to be higher than that normally expected for red meat, albeit RGBC products were the only offal type tested for STEC.

E. coli O157:H7 was not recovered from any of 1082 frozen boneless beef samples but was isolated from 0.09% of carcases examined by Philips (Philips et al, 2012). The FSIS (USDA, 2018) reported six *E. coli* O157:H7 positives from 3,238 (0.19%) routine verification samples and 0.25% prevalence of non-O157 STEC in the first 10 months of 2018. Data on the prevalence of *E. coli* O157:H7 and non-O157 STEC is collected monthly by the Australian Department of Agriculture. This data if made available could be compared with the isolation rates for STEC found in this study.

Coagulase positive (CP) *Staphylococcus* was isolated from around 10% of offal samples in the current study. No one offal type stood out as being particularly prone to contamination. For beef offal tendons were frequently contaminated (20.4%), while lamb tongues were the most frequently contaminated (34.6%). RGBC were generally the least contaminated of the offal types. Previous studies have reported 3.4% of boneless beef samples (Phillips et al, 2012) and 28.1% of retail ground beef (Phillips et al, 2008) to be contaminated with CP *Staphylococcus*. Desmarchelier et al (1999), reported between 11% and 60% of post-chill carcases positive for CP *Staphylococcus*; workers hands were found to be a source of CP *Staphylococcus* on carcases and chilling resulted in increased prevalence and numbers. The human health significance of CP *Staphylococcus* contamination of meat is questionable. In the study by Desmarchelier et al (1999) CP Staphylococcus were isolated from carcases immediately after hide removal before handled by workers. This suggests that the animals can be a source of CP *Staphylococcus*. Staphylococci are ubiquitous in nature, although commonly found on the skin and mucous membranes of mammals and birds. A large proportion of animal isolates are not associated with human illness (Vanderlinde et al, 1999). While in itself the level of Staphylococcus on fresh meat is not a food safety risk, as these bacteria do not compete well with the normal flora on red meat, they can act as a source of contamination to other foods and present a risk in processed foods that are not subjected to a bactericidal process i.e. fermented meats. Also, anecdotally, there are some customers that impose criteria for Staphylococcus on meat viewing the presence of this organism as an indicator of cross-contamination from workers during processing, ignoring the fact that the animal can be a source of contamination. Further work is needed to determine the significance of Staphylococcus levels on some red meat offal identified in the current study.

C. perfringens was isolated from only 0.7% of offal samples tested, mainly sheep samples (2.5%). In a Korean study (Im et al, 2016), *C. perfringens* was isolated form 7.1% of beef offal sampled. It is unsurprising that *C. perfringens* was found on some offal samples as this organism is ubiquitous in animals and the environment. No comment can be made on the significance of the low observed prevalence with respect to food safety except to say that offal should pose no greater risk than other meat types.

7 Conclusions

The microbiological quality of offal varied between offal type and processing establishment and while the APC and prevalence of *E. coli* were higher than typically found on meat, the average levels could be considered acceptable and consistent or better than those reported for similar products in the literature. However, given there are some high counts observed in this study, individual plants should consider whether the range of APC and *E. coli* levels for certain offal types does not put them at greater risk in the market. Pathogens were also isolated at a higher rate than would be expected for meat but not at a concerning level. Extrapolating the results of this work to the whole industry should be carried out with caution as plants participated on a voluntary basis and made up only about one third of all export registered establishments.

8 Recommendations

Based on the observations in this study, the follow recommendations are made:

1. Obtain appropriate industry data from the Department of Agriculture for the time period of the survey so that realistic comparisons between offal and meat can be made. If possible, this should be carried out at a plant level so that variability between plants can be ignored. Similarly, individual plants may wish to contribute additional in-house data for comparison purposes.
2. Investigate sources and possible interventions for pathogen contamination of RGBC offal, focusing on head meat; particularly with regards to *Salmonella* and *E. coli* O157:H7. If one or more of the participants that had detections was willing to be the focus of a case study, this could prove beneficial.

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10 Appendix: Distribution of APC on different offal types for each of the four species (beef, sheep, lamb and goat)